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Basic Amino Acid Transport in *Escherichia coli*: Properties of Canavanine-Resistant Mutants

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A mutant of *Escherichia coli* strain CanR 22 has been isolated which is resistant to growth inhibition by canavanine, an analogue of arginine. The properties of this strain and of another canavanine-resistant mutant, JC182-5 (isolated by Celis et al. [5]), were studied. The mutation is pleiotropic in that it results in a reduction in the activity of two distinct permeases, the arginine-specific and lysine-arginine-ornithine transport systems. The lesion maps at min 56 of the *E. coli* linkage map, at or near the *arg*P locus. Although strain CanR 22 excretes arginine, this excretion appears to result from reduced ability to concentrate arginine, rather than the loss of transport ability being the result of excretion. This conclusion is based on findings with a canavanine-resistant strain auxotrophic for arginine, which exhibits transport properties similar to those of the prototrophic strains. Additionally, growth in the presence of arginine or ornithine results in a repression of the activity of the two basic amino acid transport systems. Neither the arginine-specific nor the lysine-arginine-ornithine binding proteins of the mutant cells show significant alterations in terms of amount, physical properties, or kinetic parameters. These observations lead to the proposal of a model for the two basic amino acid transport systems in which two carrier proteins with different specificities interact with a common energy coupling mechanism. A lesion in the gene (or one of the genes) for this coupling mechanism can confer canavanine resistance.

The uptake of the basic amino acids arginine, lysine, and ornithine is mediated by three distinct transport systems in *Escherichia coli*, the arginine-specific system (18, 19), the lysine-arginine-ornithine (LAO) system (18), and the lysine-specific system (18). Although arginine is a substrate of LAO binding protein, which has been linked to the LAO transport system, it appears to be solely a competitive inhibitor of the LAO system (18). The transport of arginine itself is accomplished primarily via the arginine-specific system and not by the LAO system (18, 19). Precursors of arginine, such as ornithine and citrulline, and arginine analogues, such as canavanine, are transported by the LAO system (18, 19).

Canavanine has been shown to be an inhibitor of growth in *E. coli* through incorporation into protein in the place of arginine (22). Three classes of canavanine-resistant mutants have been found. One class of mutants selected for resistance to canavanine shows alterations in the regulation of arginine biosynthesis (13). A second type of resistant mutant has an altered arginyl-transfer ribonucleic acid synthetase (7). A third type of canavanine-resistant mutant isolated by Schwartz et al. shows a simultaneous loss of transport ability for the basic amino acids arginine, lysine, and ornithine (23). This observation is unexpected since canavanine is transported by the LAO system but not by the arginine-specific system (18, 19). Thus, the mutation resulting in resistance to canavanine appears to be affecting two different transport systems.

It was of interest, therefore, to study the properties of several canavanine-resistant mutants to determine the nature of the lesion. Two such mutants, one isolated in the author's laboratory, were investigated in terms of the specificity of the lesion, the kinetic properties altered, and the effect of the lesion on the arginine-specific and LAO-binding proteins. The mutation appears to affect the ability of the cells to accumulate the basic amino acids. A

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model consistent with the data is proposed in which the arginine-specific and LAO transport systems share a common step past the level of the recognition and binding site of the two systems.

MATERIALS AND METHODS

Media and growth of cultures. The minimal medium used was that described by Tanaka et al. (25), supplemented with 0.4% glycerol and 1 μg of thiamine per ml. Medium ALF (arginine and lysine free) was the same medium supplemented with essential nutrients, as described by Novick and Maas (16). For solid media, agar (Difco) was added to 2%, final concentration. Cells were grown with gyratory shaking at 37 C to mid- or late exponential phase, except for the large-scale cultures used for the isolation of binding proteins, which were grown to stationary phase in 15 liters of medium in 5-gallon (20 liters) carboys with vigorous aeration. Growth was followed by optical density measured either with a Gilford spectrophotometer at 600 nm or a Klett-Summerson colorimeter equipped with a no. 66 filter. One hundred and thirty-six Klett units is equivalent to an optical density of 1.25 and a cell concentration of 10^9 cells/ml.

Bacterial strains. All strains used are derivatives of *E. coli* K-12. Table 1 lists their relevant properties.

Chemicals. L-[*3-*^{3}*H*]Arginine, L-[*U-*^{14}*C*]arginine, L-[*U-*^{14}*C*]ornithine, L-[*4,5-*^{3}*H*]lysine, and Aquasol were purchased from New England Nuclear Corp. D,L-[*guanido-*^{14}*C*]canavanine was purchased from Schwartz/Mann. Nonradioactive amino acids were purchased from Sigma Chemical Co. All other chemicals were analytical grade. All amino acids were of the L-form unless otherwise specified.

Isolation of strain CanR 22. Strain 7 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the method of Adelberg et al. (1). A portion of the mutagenized culture was inoculated into medium ALF containing 100 μg of arginine per ml and allowed to grow to mid-exponential phase. The culture was then washed twice with minimal medium and suspended in the original volume of medium ALF. A portion of this culture was inoculated into medium ALF containing 100 μg of canavanine sulfate per ml, incubated for 4 h at 37 C, and plated onto solid medium ALF containing canavanine sulfate at the same concentration. After 24 h, colonies were picked with sterile toothpicks and streaked onto minimal plates for the isolation of single colonies. This procedure was repeated. Several single-colony isolates were then checked for canavanine resistance.

Transduction methods. The generalized transducing bacteriophage 363 was used for transduction experiments as described by Glansdorff (10).

Assay for arginine excretion. A strain of *E. coli* auxotrophic for arginine was grown to late exponential phase in minimal medium supplemented with 100 μg of arginine per ml, washed twice with minimal medium, and suspended at 10^9 cells/ml in the same medium. A 1.0-ml amount of the cell suspension was added to 10 ml of medium (at 45 C) containing 2% agar, mixed, and immediately poured onto a petri dish containing 10 ml of solidified minimal agar. After the agar hardened, suspected excreter strains were spotted on the plate and then incubated overnight at 37 C. The top layer of agar exhibited a cloudy halo around excreter strains.

Assay for transport activity. The assay for transport activity was performed as described previously (18). Cells in mid- or late exponential phase were harvested by centrifugation. Cultures were washed two times with minimal medium followed by resuspension in the same medium. Five minutes prior to the start of the assay, chloramphenicol was added at 100 μg per ml (final concentration). After 3 min, glucose was added to 20 mM (final concentration). To initiate the reaction, a portion of the cells was added to medium containing chloramphenicol, glucose (at the same concentrations specified above), and isotopically labeled amino acid (and, if indicated, unlabeled competitor). The reaction volume was 0.5 ml, and the temperature was 23 C. Portions (0.2 ml) were withdrawn at 15 and 30 s and filtered on nitrocellulose filters (25 mm, 0.45-μm pore size) (Matheson-Higgins Co., Inc., Woburn, Mass.). The filters were washed with 10 ml of standard wash medium (2), dried, and counted by liquid scintillation counting in a solution

TABLE 1. *Characterization of bacterial strains*[a]

Strain	Sex	Relevant genetic loci	Response to canavanine	Other information
7	Hfr		S	Obtained from E. C. C. Lin (11)
CanR 22	Hfr		R	Derived from strain 7 after NG treatment
JC182-5	Hfr	*thi*$^-$, *aden*$^-$	R	Obtained from W. K. Maas
MA176	F$^-$	*thi*$^-$, *leu*$^-$, *thr*$^-$, *gly*$^-$ *serA*$^-$, *lysA*$^-$	S	Obtained from W. K. Maas
MA177	F$^-$	*thi*$^-$, *trp*$^-$, *pro*$^-$, *argE*$^-$, *serA*$^-$, *speB*$^-$	S	Obtained from W. K. Maas
MA177-22	F$^-$	*thi*$^-$, *trp*$^-$, *pro*$^-$, *argE*$^-$, *speB*$^-$	R	Isolated after transduction with strain CanR 22 as donor and strain MA177 as recipient

[a] Gene coding for enzymes in various biosynthetic pathways are denoted as follows: *thi*, thiamine; *aden*, adenosine; *leu*, leucine; *thr*, threonine; *gly*, glycine; *ser*, serine; *lys*, lysine; *trp*, tryptophan; *pro*, proline; *arg*, arginine; *spe*, spermidine. Abbreviation: NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; S, sensitive; R, resistant.

consisting of 15 g of 2,5-diphenyloxazole and 0.2 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene dissolved in 3.8 liters of toluene. The 15-s values were used for the calculation of the initial rate, which is expressed as nanomoles of substrate transported per minute per milligram of cellular protein.

Osmotic shock. Osmotic shock was performed by the procedure of Neu and Heppel (15), modified for the isolation of binding proteins (18). Cultures were grown to stationary phase instead of exponential phase. In stage I of the shock procedure the concentration of ethylenediaminetetraacetic acid was increased from 0.2 to 2 mM; and in stage II, cold deionized water was used in place of 0.5 mM $MgCl_2$.

Assay for binding activity. Binding activity was measured by three methods. A filter assay, described previously (18, 19), was used to detect the emergence of binding activity from columns. A more quantitative assay, equilibrium dialysis, was used as described previously (18), with binding chambers prepared according to the specifications of Furlong et al. (9). The rapid dialysis method of Colowick and Womack (6) was used for the determination of dissociation constants as described in detail previously (19).

Polyacrylamide gel electrophoresis. Analytical polyacrylamide electrophoresis was performed in a vertical gel apparatus by using a 2-mm thick gel. A 6-cm gel consisting of 7.5% acrylamide formed the resolving gel, with a 1-cm layer of 2.5% acrylamide forming the concentrating gel. The buffer system used was that described by Davis (8). Electrophoresis was carried out until the tracking dye, bromophenol blue, approached the bottom of the slab. The gel was then fixed with 12.5% trichloroacetic acid, stained for 30 min in a solution of 0.05% Coomassie blue in 12.5% trichloroacetic acid, and destained in a solution of 7.5% acetic acid.

Other methods. The preparation of antisera against the LAO and arginine-specific binding proteins has been described in a previous report (19). Protein values were determined by a micromodification of the method of Lowry et al. (12), with bovine serum albumin as a standard.

RESULTS

Growth inhibition by canavanine. No inhibition of growth was observed when strain 7 was grown in minimal medium supplemented with up to 250 μg of canavanine sulfate per ml. When the cells were grown on medium ALF plus 100 μg of arginine per ml, followed by growth in ALF medium plus 100 μg of canavanine sulfate per ml, a marked inhibition of growth occurred after one doubling (Fig. 1). Canavanine-resistant colonies appeared after overnight incubation on plates consisting of ALF medium plus canavanine sulfate. Such mutants grow with a normal doubling time on canavanine, as shown in Fig. 1 with CanR 22.

Amino acid transport in CanR 22. The uptake of amino acids was investigated in one such canavanine-resistant mutant, CanR 22. The transport of arginine in this strain was greatly reduced in comparison with the parent organism (Fig. 2A). The accumulation of lysine and ornithine was likewise reduced, but not to the same extent (Fig. 2B and C). The small amount of arginine taken up by CanR 22 at 23 C was not significantly different than the amount taken up by strain 7 at 4 C (Fig. 2A), indicating an impaired ability to concentrate arginine. In the case of lysine transport, there is a second system, the lysine-specific transport system (18), which was found to be unaltered in CanR 22. Transport of glycine, an amino acid not involved in basic amino acid transport, was also unimpaired in this strain (Fig. 2D). Thus, the mutation appears to affect only basic amino acid transport and, specifically, the arginine-specific system and the LAO system.

Although the loss of the transport system for an analogue can confer resistance, a number of other types of lesions can likewise do so. Specifically, if the arginine biosynthetic pathway were derepressed, the internal concentration of arginine would increase, leading to resistance due to the competition of internal arginine with canavanine (13).

In such a situation, the cells might excrete arginine, leading to a dilution of the exogenous arginine in the transport assay and an apparent reduction in the activities of the arginine-specific and LAO systems, but not of the lysine-specific nor glycine systems. Indeed, all canavanine-resistant mutants isolated exhibited halos on excreter plates, whereas strain 7 did not do so. To determine whether the reduction in transport activity was due to excretion, the gene for canavanine resistance was transduced from CanR 22 into MA177, which is auxotrophic for arginine as well as *serA*$^-$. One transductant, MA177-22 (*seraA*$^+$, CanR), was examined for basic amino acid transport. This strain shows a drastic reduction in arginine transport and lesser reductions in ornithine and lysine transport (Fig. 3). However, the uptake of the basic amino acids in MA177 was itself considerably lower than that of strain 7. Since MA177 was grown in media supplemented with ornithine, it was of interest to determine the effect of such growth on strain 7. Growth in the presence of ornithine markedly reduced the transport activity of the cells for arginine, lysine, and ornithine (Fig. 4). At the concentrations of substrates used in the experiment described in Fig. 4, essentially all of the arginine is transported by the arginine-specific system, and greater than 80% of the lysine is transported by the LAO system. Additional experiments (data not shown) at 10 μM lysine show that arginine-insensitive lysine transport is not af-

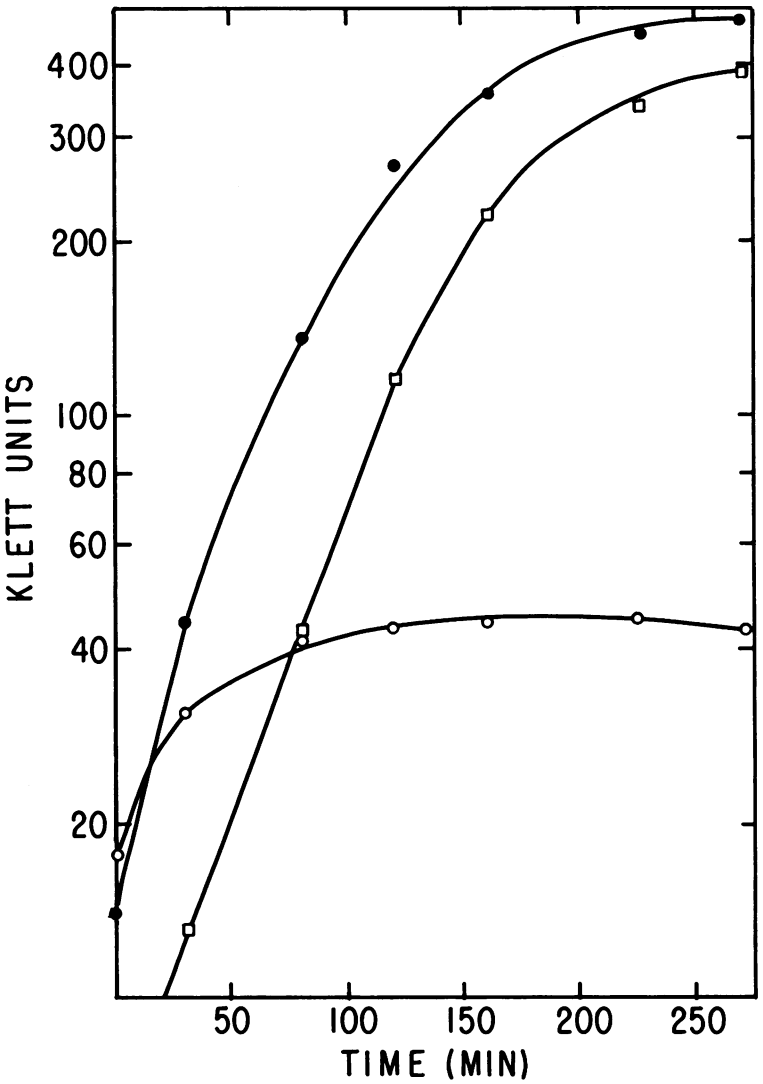

FIG. 1. *Growth of parent and mutant strains in media containing canavanine. Cells were grown in medium ALF supplemented with 100 μg each of arginine and lysine per ml to exponential phase. The cells were collected by centrifugation, washed twice with minimal medium, and suspended in medium ALF supplemented with either 100 μg of canavanine sulfate or arginine per ml. Symbols: ●, strain 7 plus arginine; ○, strain 7 plus canavanine; □, strain CanR 22 plus canavanine.*

fected by growth of the cells in ornithine. It appears, then, that the arginine-specific and LAO systems are repressed by growth in ornithine. Growth of strain 7 in the presence of arginine was found to have a similar affect, although growth in the presence of lysine caused no such repression of arginine-specific or ornithine transport but did, however, repress the lysine-specific system. Celis et al. (5) have likewise found these repressive effects and have made some additional observations concerning them.

A similar phenomenon has been found in the case of the two leucine transport systems (17).

Yet, it does appear that the reduction in basic amino acid transport in CanR 22 is a result of the loss of permease activity as opposed to an apparent reduction in activity due to excretion. Since CanR 22 cannot actively accumulate arginine to any great extent (the concentration ratio is less than 10 compared to approximately 2,000 for strain 7), it may also not be capable of retaining its arginine pool, leading to a passive excretion of arginine.

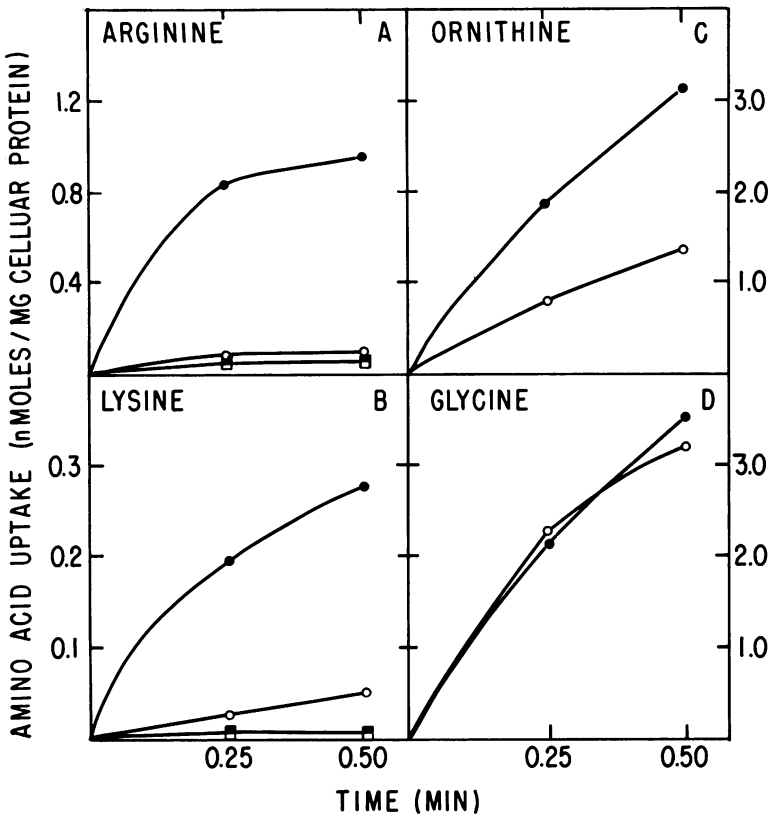

FIG. 2. *Amino acid uptake in strains 7 and CanR 22. Cultures grown in minimal medium were used for uptake assays as described in Materials and Methods. (A) Uptake of [^3H]arginine at 0.25 μM arginine, initial concentration. (B) Uptake of [^3H]lysine at 0.25 μM lysine, initial concentration. (C) Uptake of [^{14}C]ornithine at 10 μM, initial concentration. (D) Uptake of [^{14}C]glycine at 10 μM, initial concentration. Symbols: ●, strain 7, 23 C; ○, strain CanR, 23 C; ■, strain 7, 4 C; □, strain CanR 22 4 C.*

Kinetics of arginine transport in canavanine-resistant mutants. It was of interest to determine whether the lesion resulting in canavanine resistance affected the affinity of the arginine-specific and LAO transports systems, the maximal velocities, or both. Due to the low initial rates of basic amino acid transport in such mutants, it was impossible to obtain accurate kinetic data. The results of numerous assays of arginine transport at varying concentrations of arginine show a definite decrease in the V_{max} of the arginine-specific transport system in both CanR 22 and JC182-5. The data were not accurate enough to obtain K_m values for the mutants.

The transport of canavanine itself is greatly reduced in CanR 22 as compared to strain 7. Figure 5 shows the concentration dependence of canavanine uptake on canavanine concentration. The K_m in strain 7 is approximately 0.4 mM. The values from assays of CanR 22 were too low to allow for the determination of a kinetic constant.

Mapping of the gene yielding the canavanine-resistant phenotype. Previous experiments by Maas (14) have localized the gene responsible for canavanine resistance at min 56 on the linkage map of the *E. coli* chromosome. This locus has been termed the *argP* locus (14). The gene responsible for the canavanine-resistant phenotype in CanR 22 was localized by transduction (Table 2). Here strain CanR22 (*serA*$^+$*speB*$^+$*canR*) was crossed as donor with strain MA177 (*serA*$^-$*speB*$^-$*canS*) as recipient. *serA*$^+$ transductants were selected. The cotransduction frequencies with *serA*$^+$ were 96% for the canavanine resistance locus and 50% for *speB*. If the *canR* locus were on the other side of *serA* from *speB*, transductants of the class *canS argS* would require a double cross-over, whereas those of the class *canS argR* would require a single cross-over. The reverse would be true if the *canR* locus were on the same side of *serA* as *speB*. Since no transductants of the class *canS argR* were found, it is likely that the *canR* locus is on the same side of *serA* as *speB*. This is in

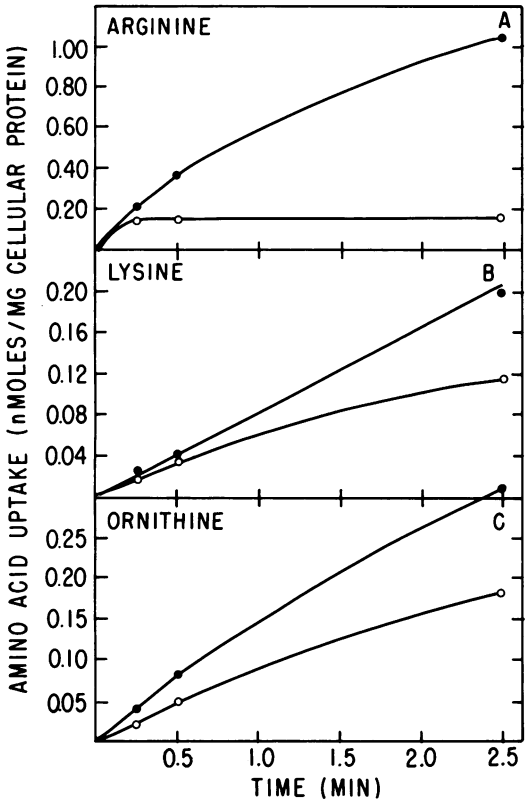

FIG. 3. *Amino acid uptake in strains MA177 and 177-22. Cultures were grown in minimal medium supplemented with 100 μg of arginine per ml. Uptakes were performed as described in Materials and Methods. The concentrations of radioactive amino acids were the same as given in Fig. 2. Symbols: ●, strain MA177; ○, strain MA177-22.*

agreement with the results of Maas (14).

When strain MA176 (*lysA⁻serA⁻canS*) was the recipient with CanR 22 (*lysA⁺serA⁺canR*) again as the donor, no transductants of the genotype *lys⁺ser⁺canS* or *lys⁺ser⁻canR* were found. Of 200 *lysA⁺* transductants, 181 were *ser⁺canS* and 19 were *ser⁺canR*. Thus, both *serA* and *canR* have co-transductions frequencies of about 10% with *lysA* and 100% with each other. These results are again in agreement with those of Maas (14). For this reason it appears likely that the lesion in CanR 22 is in the *argP* locus or a locus which is closely located to the *argP* gene.

Arginine-specific and LAO binding proteins of canavanine-resistant strains. Previous work in this laboratory has indicated the involvement of the shock-releasable arginine-specific and LAO binding proteins in the transport of the basic amino acids. It was of interest, therefore, to determine the effect of *argP* mutations on these proteins. The osmotic shock fluids from strains CanR 22 and JC182-5 were chromatographed on diethylaminoethyl (DEAE)-cellulose, with the arginine binding fractions measured by the filter assay. The effluents from both strains contain all three arginine binding proteins (18), with peaks 2 and 3 corresponding to the LAO and arginine-specific binding proteins, respectively. The ionic strength necessary to elute these proteins is identical to that required in strain 7 (18). The amounts of the two binding proteins in the canavanine-resistant strains is similar to those of strain 7, although JC182-5 appears to contain a lower level of the arginine-specific binding protein (Table 3).

Likewise, the DEAE fractions exhibit protein bands with the identical mobilities of the purified binding proteins from strain 7 and also show the same reaction to the antisera prepared against the purified proteins from strain 7. The dissociation constants of the LAO binding proteins from all three strains are nearly identical. The purified LAO binding protein from strain 7

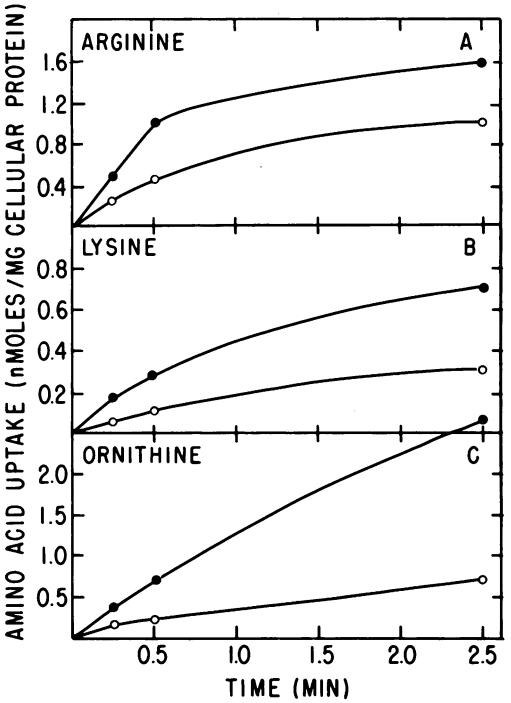

FIG. 4. *Amino acid uptake in strain 7 grown in the presence or absence of ornithine. Cultures were grown in minimal medium without amino acid supplementation (●) or in the presence of 100 μg of ornithine (○) per ml. Uptake assays were performed as described in Materials and Methods, with conditions as described in Fig. 1.*

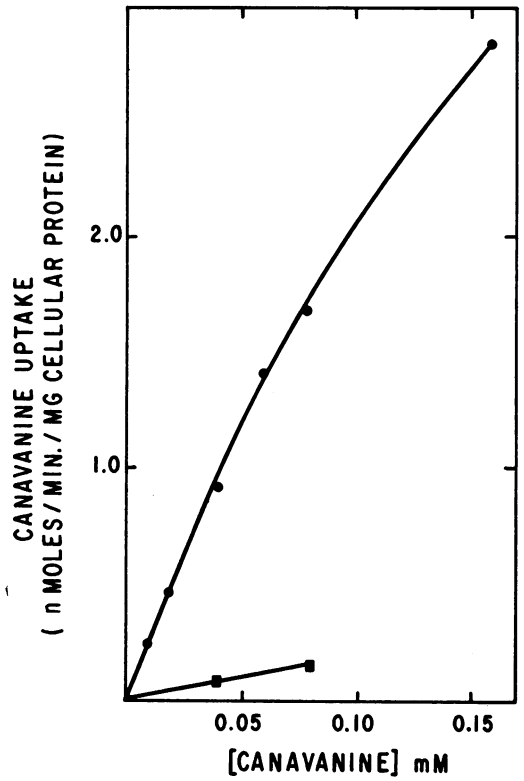

FIG. 5. *Concentration dependence of canavanine uptake in strains 7 and CanR 22. Uptakes were performed as described in Materials and Methods, with using varying concentrations of* [^{14}C] *canavanine. Symbols:* ●, *strain 7;* ■, *CanR 22.*

TABLE 2. *The order of the speB and argP genes with respect to serA*[a]

Classes	Distribution of unselected markers	
	No.	Frequency (%)
CanR ArgS	23	46
CanR ArgR	25	50
CanS ArgS	2	4
CanS ArgR	0	0

[a] *SerA*$^+$ progeny were selected in a transduction with strain CanR 22 (*serA*$^+$*speB*$^+$*argP*$^-$) as donor and strain MA177 (*serA*$^-$*argP*$^+$*speB*$^-$) as recipient. The phenotype of *argP*$^-$ mutants can be recognized by resistance to canavanine, whereas those of *speB*$^-$ are sensitive to arginine.

has a K_d for arginine of 0.2 μM; the LAO binding protein from CanR 22 has a K_d of 0.4 μM for the same substrate; and the dissociation constant of that protein from JC182-5 is 0.2 μM. Likewise, the three arginine-specific binding proteins exhibit similar binding constants: strain 7, K_d = 0.03 μM (19); CanR 22, K_d = 0.06 μM; and JC182-5, k_d = 0.04 μM. Thus, with respect to the basic amino acid binding proteins, there is no difference between the canavanine-sensitive and canavanine-resistant strains.

DISCUSSION

At least three classes of mutations affecting permeation have been isolated in various laboratories. One class is illustrated by the work of Boos (4), in which an alteration in the structural gene for a binding protein results in an alteration in the K_m of the transport system. The second class appears to be regulatory in nature. An example is provided by *glnP1*, a derivative of strain 7 which has an increased amount of glutamine binding protein and a corresponding increase in the rate of glutamine transport (26). The important aspect for comparison with this study is that, while the initial rate of transport is elevated, the concentration ratio is the same as that of the parent organism (27). Other mutants of the same class have reduced amounts of binding protein and reduced rates of transport (3). The transport reaction has been shown to obey Michaelis-Menten kinetics, where the initial rate, v_{influx}, is dependent on the external substrate concentration, S_e, so that

$$v_{\text{influx}} = \frac{S_{(e)} V_{max(e)} Q}{S_{(e)} + K_{m(e)}} \quad (1)$$

where $K_{m(e)}$ is the affinity of the carrier on the external side of the membrane, $V_{max(e)}$ is proportional to the quantity of carrier proteins per unit area of membrane, and Q is the efficiency of the energy coupling mechanism (24). Thus, a regulatory mutant in which the number of carrier

TABLE 3. *Quantitation of basic amino acid binding proteins*[a]

Strain	LAO binding protein (U[b]/g of wet cells)	Arginine-specific binding protein
7	1.62[c]	1.71[c]
CanR 22	1.18	1.34
JC182-5	1.03	0.72

[a] The peaks from DEAE-cellulose containing the arginine-specific and LAO binding proteins were concentrated by Amicon ultrafiltration and assayed for binding activity by equilibrium dialysis, as described in Materials and Methods. [^3H]Arginine was used as substrate at 10 μM initial concentration.

[b] One unit of binding activity is equivalent to 1 nmole of protein-substrate complex formed.

[c] Data from reference (18).

proteins were altered would lead to an alteration in the V_{max}. It should be noted that the value obtained for the maximal velocity in a reciprocal plot of equation 1 is an apparent V_{max}, equivalent to the quantity $V_{max(e)}\ Q$. If efflux occurs by the same system, but with the effect of energy coupling being to lower the affinity of the carrier on the inside of the membrane, then efflux would be given by

$$v_{efflux} = \frac{S_{(i)}V_1}{S_{(i)} + K_{m(e)}} + \frac{S_{(i)}V_2}{S_{(i)} + K_{m(i)}} \quad (2)$$

Here, $S_{(i)}$ is the internal concentration of substrate, $K_{m(i)}$ is the affinity of the carrier after modification by the input of energy, and V_1 and V_2 are related to the proportion of carrier molecules in the unaltered and altered states, respectively. Under normal conditions, it is expected that V_1 would be negligible, so that the relation would reduce to

$$v_{efflux} = \frac{S_{(i)}V_{max(i)}}{S_{(i)} + K_{m(i)}} \quad (3)$$

The concentration ratio, CR, at the steady state would be

$$CR = \frac{S_{(i)}}{S_{(e)}} = \frac{[S_{(i)} + K_{m(i)}][V_{max(e)}]\,Q}{[S_{(e)} + K_{m(e)}][V_{max(i)}} \quad (4)$$

It is apparent that CR is independent of the number of carrier molecules and implies that the time necessary to attain the steady state would increase with decreasing concentration of carrier molecules. Likewise, in a mutant such as *glnP1*, where the number of molecules of glutamine binding protein per cell is increased, the time necessary to attain the steady state would decrease with no change in the concentration ratio, assuming that the glutamine binding protein is the carrier protein of the glutamine transport system. Evidence for, and speculation on, the relationship of such binding proteins to transport have recently been reviewed (20). This effect is simulated by the osmotic shock procedure, where the K_m is unaffected and the apparent V_{max} is reduced. In a carefully controlled experiment, the concentration ratio remains the same (B. P. Rosen and F. D. Vasington, Fed. Proc., p. 342, 1970).

These considerations lead to an explanation of the third class of permease mutation, that in which the carrier protein has become uncoupled from the concentrative apparatus. In such a situation, one would expect a decrease in the apparent V_{max} as a result of an alteration in Q. This would also lead to a decrease in the concentration ratio, with no alteration in the concentration or binding constant of the carrier molecules. Moreover, in such a mutant the number of carrier molecules on the inside of the membrane in the low-affinity state would be reduced, with a corresponding increase in the number of carriers in the high-affinity state. So, from equation 2, the velocity of efflux would be greater at any subsaturating internal concentration of substrate, leading to excretion of the biosynthetic pool of substrate. This type of phenotypic expression might be the result of a lesion in the gene for the binding protein, as has been postulated for certain uncoupled mutants in the lactose transport system (28), or might be an alteration in some other step of the coupling mechanism.

In the case of the canavanine-resistant strains discussed above, it would seem likely that the mutation is in one of the genes of the energy coupling step for the following reasons. First, the concentration ratio is reduced approximately 200-fold. Second, the mutation affects two separate transport systems, suggesting a common protein to which the carrier proteins of both the LAO and arginine-specific systems couple. Third, the mutant excretes arginine, as would be expected for an energy-uncoupled system. Finally, neither the amount nor the properties of either the LAO or arginine-specific binding proteins are altered. The following hypothesis is consistent with all of the above observations. (i) The arginine-specific binding protein is the carrier protein of the arginine-specific system and binds only arginine. (ii) The LAO protein is the carrier molecule of the LAO system and has affinity for arginine, lysine, and ornithine. (iii) Both proteins are bifunctional, with the filling of the substrate site leading to a conformational change in a second site. (iv) The "correct" conformation of this second site permits the protein-substrate complex, in turn, to be a substrate of the energy-coupling protein(s). A single coupling protein or a single set of proteins would act on both carrier proteins. The fact that arginine is a substrate of the LAO binding proteins, but only an inhibitor of the transport system, leads to the conclusion that the arginine-LAO protein complex does not have the correct conformation at the second site. The loss of this coupling system confers resistance to canavanine, a substrate of the LAO system, and simultaneously causes the loss of the arginine-specific transport system of which canavanine is not a substrate. Although they are not unique to this model, one can predict that it should be possible to isolate strains of the phenotypes arginine permease positive, LAO permease and negative canavan-

line resistant and arginine permease negative LAO permease positive, and canavanine sensitive.

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