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Genetic Separation of High- and Low-Affinity Transport Systems for Branched-Chain Amino Acids in *Escherichia coli* K-12

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The *Escherichia coli* K-12 mutant strain AE4107 (*livH*::Mu) is defective in the high-affinity binding protein-mediated uptake system for L-leucine, L-valine, and L-isoleucine (LIV-I). We have used this strain to produce mutations in the residual LIV-II membrane-bound branched-chain amino acid uptake system. Mutants selected for their inability to utilize exogenous L-leucine were found to be defective in the LIV-II system and fell into two classes. One class, represented by strain AE410709 (*livP9*), showed a complete loss of saturable uptake for L-leucine, L-valine, and L-isoleucine up to 50 μM, and a second class, represented by strain AE4017012 (*liv-12*), showed a residual component of saturable leucine uptake with increased K_m. These mutations, *livP9* and *liv-12*, were closely linked and mapped in the 74 to 78 min region of the *E. coli* genetic map. Strains constructed so that they lacked both LIV-I and LIV-II transport systems excreted leucine. Strains of the genotype *livH*$^+$ *livP* were found to have normal high-affinity binding protein-mediated transport (LIV-I and leucine specific), whereas the low-affinity (LIV-II) transport was completely missing. We concluded from these studies that the high-affinity binding protein-mediated transport systems (LIV-I and leucine specific) can operate independently of the membrane-bound LIV-II system.

The transport of the branched-chain amino acids L-leucine, L-valine, and L-isoleucine in *Escherichia coli* K-12 is mediated by several kinetically and genetically distinct systems (1, 8, 9, 14, 18). The high-affinity, osmotic shock-sensitive uptake of leucine includes the LIV-I transport system requiring the LIV binding protein (*livJ*) and the leucine-specific system requiring the leucine-specific binding protein (7) (*livK*). Both binding protein systems require the product of the *livH* gene (1) and are repressible by L-leucine (13). The low-affinity membrane-bound transport system for the branched-chain amino acids has been designated the LIV-II system (14, 18). This latter system resists osmotic shock and has been observed in membrane vesicle preparations (18). We have been using genetic approaches to determine the number of components of each system and their mutual interactions. In this report we describe the isolation and characterization of mutants lacking the LIV-II system and the properties of strains which possess the high-affinity systems but are missing the membrane-bound LIV-II system.

MATERIALS AND METHODS

Bacteria and phage. The bacterial strains used were all derivatives of *E. coli* K-12 and are listed in Table 1. For transductions the phage P1CM*clr*100 was used.

Materials. Morpholinopropane sulfonic acid and nutritional supplements were obtained from Sigma Chemical Co., St. Louis, Mo.

Media. Minimal medium for growth of cultures used in transport assays was the morpholinopropane sulfonic acid-based medium (MOPS-salts) described previously (12). Amino acids and thymine were supplemented at 50 μg/ml, except for L-leucine (see below). Unless otherwise noted, glucose was used at 0.2%. Luria broth with thymine was used for routine culture and for preparing cells for genetic crosses; recombinant selective agar plates were prepared with medium 56 as described previously (2).

Genetic techniques. Cells were grown at 37°C in a New Brunswick Aquatherm shaker (New Brunswick Scientific Co., New Brunswick, N.J.), and growth was followed by measuring absorbance at 420 nm in a GCA/McPherson EU-700 spectrophotometer. Absorbance was converted to milligrams of dry weight per milliliter by using the method of Koch (10).

Culture conditions. Conjugations and P1 transductions were carried out as described by Miller (11).

Transport assays. Transport of L-^{3}H-amino acids was performed as described previously (1). Strains were grown overnight in limiting glucose (0.02%)-MOPS-salts plus required supplements, and growth was renewed by adding glucose to 0.2%. After 1.5 mass

TABLE 1. *E. coli K-12 strains*

Strain	Relevant genotype	Source
AE41	*lstR leu-6 malT1 xyl-7* F^-	Anderson et al. (2)
AE91	*lstR livH*::Mu *mal*$^+$ *leu-6*	Anderson et al. (1)
AE125	*lstR livH*::Mu *liv-12 mal*$^+$ *leu-6*	*mal*$^+$ transductant of AE4107012 from donor E0300 (K-12 wild type)
AE137	*lstR livH*$^+$ *liv-12 xyl*$^+$ *leu-6*	*xyl*$^+$ recombinant of mating between KL14 and AE4107012
AE166	*lstR livH*$^+$ *livP9 mal*$^+$	*mal*$^+$ transductant of strain AE410709 from donor E0300 (K-12 wild type)
AE4107	*lstR livH*::Mu *malT1 leu-6*	Anderson et al. (1); as AE41
AE410709	*lstR livH*::Mu *livP9 malT1 leu-6*	This paper; as AE41
AE4107012	*lstR livH*::Mu *liv-12 malT1 leu-6*	This paper; as AE41
KL14	Hfr	CGSC[a]

[a] CGSC, *E. coli* Genetic Stock Center.

doublings, the cells were harvested by centrifugation and washed. It was necessary to wash cells five times with MOPS-salts before assay to minimize the extracellular leucine required by some of the strains used in these studies. The cells were suspended in MOPS-salts plus 0.2% glucose but minus required amino acids, to prevent net protein synthesis during the course of assay. After a 10-min adaptation, assays were initiated by adding 0.4 ml of cell suspension to 0.4 ml of L-^{3}H-amino acid in MOPS-salts. A 0.4-ml sample was taken at an appropriate time, usually 10 s, and filtered through a 24-mm-diameter Millipore HA filter (0.45 μm; Millipore Corp., Bedford, Mass.), followed by a 5-ml wash of 10 mM potassium phosphate buffer, pH 7.2, at 37°C. Radioactivity was determined in a Packard liquid scintillation spectrometer, using a standard scintillation solution.

Determination of kinetic constants. For the studies reported here, biphasic kinetics were generally not observed when data were plotted by the double reciprocal method. For this simple situation we have employed the direct linear plot of Eisenthal and Cornish-Bowden (6) to determine median values of apparent K_m and V_{max} of uptake. This relatively simple graphical method avoids assumptions about the distribution of experimental error, the statistical difficulties of curve fitting to linear transformations of the Michaelis-Menton equation, and the necessity of determining weighting values over the distribution of experimental points. The use of the sample median, rather than the mean, reduces the influence of outliers, that is, aberrant observations which frequently arise in the measurement of uptake in whole cells. A further advantage of this method is in the simple determination of nonparametic confidence limits for the medium values of K_m and V_{max} (5); these kinetic values have been incorporated into the tables of this paper. Generally, we have plotted velocities from five ligand concentrations symmetrically distributed about the expected K_m value. For this number of samples, we have been able to define 68.8% confidence limits for the median estimates of the K_m and V_{max} values (5).

Isolation of mutants altered in the LIV-II system. Strain AE4017 (*livH*::Mu) was mutagenized with ethyl methane sulfonate as described by Miller (11). The cells were washed twice in MOPS-salts and suspended in MOPS-salts supplemented with 0.02% glucose and 20 μg of L-leucyl-L-alanine per ml as a source of L-leucine that could enter the cells independent of the LIV-I and LIV-II uptake systems (9). This technique avoided counterselecting mutants lacking both systems. After overnight growth at 37°C, glucose was added to 0.2%, and recovery was permitted for 1 mass doubling. The cells were washed by centrifugation three times and suspended in MOPS-salts supplemented with 0.2% glucose and 11.4 μM L-leucine. This level of L-leucine is below saturation of the residual LIV-II uptake system in strain AE4107. An initial cell density was chosen (absorbance at 420 nm, 0.04) which prior experiments showed would sustain 1.5 generations of logarithmic growth before L-leucine exhaustion. Ampicillin (final concentration, 20 μg/ml; Amcill-S; Parke, Davis & Co., Detroit, Mich.) was added immediately, and growth was permitted for 2 h at 37°C. Cells and cell debris were sedimented at 20,000 × *g*, washed once, and suspended in 0.02% glucose–MOPS-salts supplemented again with 20 μg of L-leucyl-L-alanine per ml. Another cycle of ampicillin enrichment was performed, the cell pellet was resuspended, and dilutions were plated on Luria broth with thymine. Colonies were replicated to glucose minimal agar containing either 3 μg of L-leucine per ml or 20 μg of L-leucyl-L-alanine per ml. Colonies showing growth on the latter plate but not on the former were cloned and checked for transport of L-leucine and L-alanine simultaneously as described previously (1). The yield of mutants with normal L-alanine transport but reduced L-leucine transport was 2%. Representatives of two phenotypic classes (strains AE410709 and AE4107012) were chosen for further study.

RESULTS

Mutants defective in the LIV-II uptake system. Two phenotypically distinguishable mutants unable to grow on 3 μg of L-leucine per ml were isolated as described above. Table 2 shows that they differ in their ability to utilize exogenous L-leucine. Strain AE410709 requires a relatively high (100 μg/ml) plate concentration of L-leucine to grow, and strain AE4107012 grows well on one-third (30 μg/ml) this level of leucine. We have designated the new mutant locus in

strain AE410709 as *livP9* and use it as the type strain for this locus. In the absence of evidence that the mutation in strain AE4107012 is allelic, we have designated this mutation as *liv-12*. Both mutants have specific defects in leucine uptake, since the transport of other amino acids has not been altered (Table 3) relative to the parent strain AE4107.

Figure 1 shows the kinetics of L-leucine, L-valine, and L-isoleucine uptake in both mutant strains. The uptake of L-leucine, L-valine, and L-isoleucine is severely depressed relative to the parent strain AE4107 (Fig. 1 and Table 3) and in strain AE410709 (*livP9*) fails to demonstrate saturability up to 50 μM L-leucine. Strain AE4107012 (*liv-12*) differs only in that a minor saturable component for L-leucine is detectable (Fig. 1 and Table 3) and presumably accounts for the lower threshold of this strain for L-leucine growth supplementation (Table 2). Because both mutants show single-step reversion (frequency, approximately 10^{-8}) and are readily transduced to *livP*$^+$, we infer that single mutations are responsible for the observed phenotypes and that these mutations specifically affect the residual LIV-II transport system of the parent strain AE4107.

Strains AE410709 (*livP9*), AE4107012 (*liv-12*), and AE4107 (*livP*$^+$) were transduced to *leu*$^+$ and tested for excretion of L-leucine by spotting the strains on a lawn of strain AE41 (*leu*). After a 3-day incubation at 37°C, both mutant strains showed cross-feeding, but the parent (*livP*$^+$) did not. We conclude that the loss of both LIV-I and LIV-II systems resulted in a minor loss of ability to retain internal L-leucine.

Properties of the LIV-I system in the absence of the LIV-II system. Because *livH*$^+$ is cotransducible with *mal*$^+$ (1) (Fig. 2) and *livP9* is not (see below), it was possible to construct strains carrying either *livP9* or *liv-12* mutations in a *livH*$^+$ background, which would allow the LIV-I system to be studied in the absence of the LIV-II system. Table 2 shows that strains AE137 (*livH*$^+$ *liv-12*) and AE166 (*livH*$^+$ *livP9*) have regained the ability to grow on low levels of L-leucine, as well as the ability to use D-leucine as a source of L-leucine, a property requiring the high-affinity leucine-specific transport system (1). Figure 3 shows that L-leucine uptake in strain AE166 (*livH*$^+$ *livP9*) saturates at low concentrations of L-leucine, which would be expected of the high-affinity LIV-I and leucine-specific systems operating alone and not characteristic of the LIV-II uptake system operating alone (strain AE4107 *livH*::Mu). The additive uptake of leucine through the LIV-I, leucine-specific, and LIV-II transport systems is illustrated in the wild-type strain AE41. The kinetics of strain AE137 (*livH*$^+$ *liv-12*) are similar to those for strain AE166, but we have omitted them for clarity. Table 4 shows the low K_m for L-leucine uptake of strain AE137 (*livH*$^+$ *liv-12*), as would be expected for the high-affinity LIV-I and leucine-specific systems operating in the absence of the LIV-II system. We conclude that, because the LIV-I and leucine-specific systems function normally in the absence of a functional LIV-II system, and vice versa, the binding protein-mediated systems do not require the oper-

TABLE 2. *Agar plate phenotypes of liv mutants*

Strain	Relevant genotype	Colony formation[a] with:			
		3 μg of L-Leucine/ml	30 μg of L-Leucine/ml	100 μg of L-Leucine/ml	150 μg of D-Leucine/ml
AE41	*livH*$^+$ *livP*$^+$ (wild type)	+	+	+	+
AE4107	*livH*::Mu *livP*$^+$	+	+	+	−
AE4107012	*livH*::Mu *liv-12*	−	+	+	−
AE410709	*livH*::Mu *livP9*	−	−	+	−
AE137	*livH*$^+$ *liv-12*	+	+	+	+
AE166	*livH*$^+$ *livP9*	+	+	+	+

[a] Visible colonies after 48 h at 37°C.

TABLE 3. *Specificity of transport alterations in liv strains*

Strain	Uptake (nmol min^{-1} mg^{-1} of dry wt)			
	L-Arginine[a]	L-Proline[a]	L-Phenylalanine[a]	L-Methionine[a]
AE4107 (*livH*::Mu)	3.3	0.70	0.37	0.42
AE410709 (*livH*::Mu *livP9*)	3.0	0.76	0.28	0.37
AE4107012 (*livH*::Mu *liv-12*)	3.2	0.72	0.32	0.29

[a] Amino acid concentration was 5 μM.

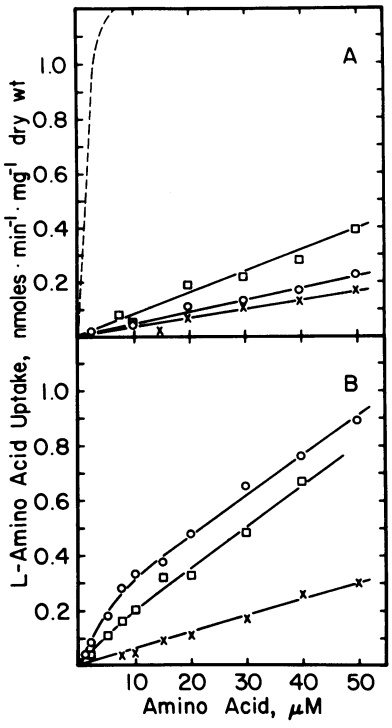

FIG. 1. *Branched-chain amino acid uptake kinetics in strains AE410709 (livH::Mu livP9) (A) and AE4107012 (livH::Mu liv-12) (B) grown in minimal medium containing 100 μg of* L*-leucine per ml. Symbols:* ○, L*-leucine;* □, L*-isoleucine;* ×, L*-valine. The dashed line in (A) shows* L*-leucine uptake of the parent strain AE4107 (livH::Mu livP⁺) for comparison.*

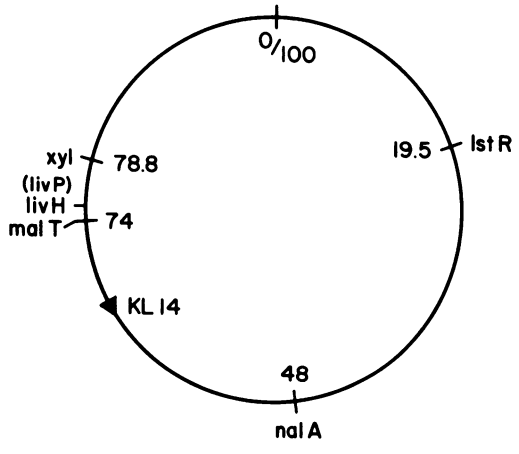

FIG. 2. *E. coli genetic map showing positions of genes referred to in this paper (3). The positions of livH and lstR have been reported previously (1, 2). The arrowhead denotes the point of origin of the Hfr KL14.*

ation of the membrane-bound LIV-II system; that is, the two types of systems can operate independently. When wild-type strains (*livH⁺ livP⁺*) or strains deficient in the high-affinity uptake systems only (*livH livP⁺*) are converted to spheroplasts with lysozyme-ethylenediaminetetraacetic acid treatment, residual uptake of leucine through the LIV-II system can be demonstrated (data not shown). However, strains of the genotypes *livH livP* or *livH⁺ livP* lose all detectable leucine uptake after conversion to spheroplasts, supporting the hypothesis that the *livP* gene product is an essential component of the osmotic shock-resistant LIV-II system.

Mapping of *livP9* and *liv-12*. Genetic mapping in strains AE410709 (*livH*::Mu *livP9*) and AE4107012 (*livH*::Mu *liv-12*) is complicated by the fact that selection or screening for the ability to grow on low levels of L-leucine does not discriminate between *livH⁺* and *livP⁺*. These complications are the result of the independent functioning of each system and the ability of each isolated system to utilize exogenous L-leucine efficiently (Table 2). Table 5 shows the results of an uninterrupted mating between the Hfr strain KL14 and the two mutant strains, where ability to grow on 3 μg of L-leucine per ml was the selected character. Ideally, the donor Hfr (strain KL14) should have carried the *livH*::Mu insertion of the recipient so that *livH* would not have been involved in the cross; however, for unknown reasons it was not possible to cotrans-

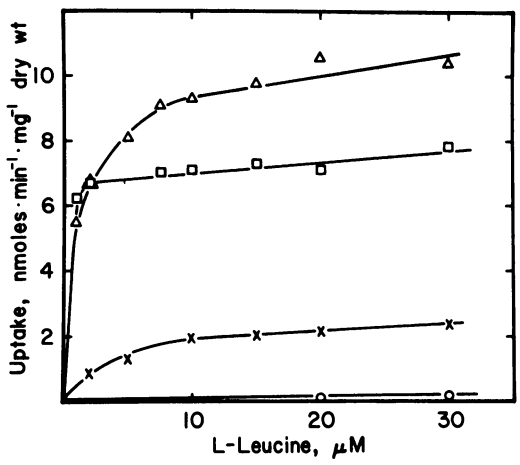

FIG. 3. L*-Leucine uptake kinetics in E. coli strains with different combinations of uptake systems:* △, *strain AE41 (livH⁺ livP⁺), containing the LIV-I and LIV-II systems;* □, *strain AE166 (livH⁺ livP9), containing the LIV-I system only;* ×, *strain AE4107 (livH::Mu livP⁺), containing the LIV-II system only;* ○, *strain AE410709 (livH::Mu livP9), containing neither LIV-I nor LIV-II systems.*

TABLE 4. *Values of kinetic parameters of uptake in liv mutants*

Strain[a]	L-Leucine		L-Valine		L-Isoleucine	
	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1} of dry weight)	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1} of dry weight)	K_m	V_{max} (nmol min^{-1} mg^{-1} of dry weight)
AE4107 (*livH*::Mu)	4.9 (2.5–6)[b]	2.4 (2–3.3)	6.8 (2–8.5)	1.7 (1.3–2.2)	5.4 (4–9)	1.65 (1.2–2.4)
AE410709 (*livH*::Mu *livP9*)	ND[c]	ND	ND	ND	ND	ND
AE4107012 (*livH*::Mu *liv-12*)	20 (15.5–27)	0.9 (0.7–1.2)	ND	ND	ND	ND
AE137 (*livH*$^+$ *liv-12*)	0.6 (0.4–0.9)	3.1 (2.5–4)	—[d]	—	—	—

[a] Strains were grown in 0.2% glucose–MOPS-salts medium plus 100 μg of L-leucine per ml; all are *leu* and *lstR*.
[b] The values are median estimates with 70% confidence intervals (values in parentheses) as determined from direct linear plots.
[c] ND, No detectable saturable uptake over a concentration range of 0.5 to 50 μM.
[d] —, Not tested.

TABLE 5. *Distribution of markers among recombinants from uninterrupted matings between Hfr KL14 and liv mutants*

Donor	Recipient	Selected character[a]	Recombinant class	% of total
KL14 *mal*$^+$ Nal^s	AE410709 (*livH*::Mu *livP9 xyl malT* Nal^r)	Leu$_3^+$ (300)	*livH*::Mu *livP*$^+$	14.1
			livH$^+$ *livP*$^+$/*P*[b]	85.9
			livH::Mu *livP*$^+$ *xyl*	10.1
			livH::Mu *livP*$^+$ *xyl*$^+$	4
	AE4107012 (*livH*::Mu *liv-12 xyl malT* Nal^r)	Leu$_3^+$ (300)	*livH*::Mu *livP*$^+$	16.5
			livH$^+$ *livP*$^+$/*P*[b]	83.5
			livH::Mu *livP*$^+$ *xyl*	11.1
			livH::Mu *livP*$^+$ *xyl*$^+$	5

[a] Colony formation on 3 μg of L-leucine per ml. This selection does not discriminate between *livH*$^+$ and *livP*$^+$ recombinants. The Hfr was counterselected with 15 μg of nalidixic acid per ml. The number tested is given in parentheses.
[b] Strains carrying *livH*$^+$ grow on 150 μg of D-leucine per ml. *livP* and *livP*$^+$ are not readily determined in this class.

duce *livH*::Mu with *malT*$^+$ in a *malT* derivative of strain KL14. When the *livH*$^+$ donor was used, two classes of recombinants were observed. Those recombinants which acquired D-leucine utilization had acquired the *livH*$^+$ allele of the donor, regardless of the disposition of *livP9* or *liv-12*, which are technically difficult to detect. The second class (*livH*::Mu *livP*$^+$) is unambiguous because these recombinants had not acquired D-leucine utilization and had retained the *livH*::Mu allele of the recipient. If interference of crossing over is not significant, an equal proportion (14% in the case of AE410709) of *livP*$^+$ can be assumed to be present in the *livH*$^+$ class of recombinants as in the *livH* class. For strain AE410709, then, the frequency of *livP*$^+$ (2 × 14.1% = 28.2%) is lower than the frequency of *livH*$^+$ (85.9%). Similarly, for strain AE4107012 the frequency of *liv*$^+$*-12* (2 × 16.5% = 33%) is lower than that of *livH*$^+$ (83.5%). Because the mating was uninterrupted, the declining gradient of transmission of markers from the point of origin of the Hfr (Fig. 2) indicates that *livP9* and *liv-12* are distal to *livH*. An interrupted mating using the same strains verified that *livP* is not located between the point of origin of strain KL14 and *malT* (data not shown). Table 5 also shows that, when the frequency of *xyl*$^+$ recombinants was assessed among the *livH*::Mu *livP*$^+$ or *liv*$^+$*-12* recombinant class, the donor marker (*xyl*$^+$) was underrepresented in the progeny (4% *xyl*$^+$ compared with 10.1% *xyl*). This suggests thtat *livP9* and *liv-12* are located between *livH* and *xyl* (Fig. 2). When P1 transducing phage were prepared on strain AE91 (*mal*$^+$ *livH*::Mu *livP*$^+$) and used to transduce strain AE410709 (*malT livH*::Mu *livP9*) to *livP*$^+$ (growth on 3 μg of L-leucine per ml) without complication from the *livH* allele, no detectable cotransduction of *mal*$^+$ was observed (<1%). Reciprocally, if *mal*$^+$ was the selected marker, *livP*$^+$ cotransductants were not observed (<1%). Identical results were obtained with strain AE4107012 as recipient. Although it appears that *livP* and *malT* are not contransducible, we recognize that *livP* and *malT* are probably bracketing the Mu insertion of the *livH*::Mu donor, a circumstance which would lower their apparent linkage by the size of the Mu genome (30% of the transducing capacity of P1). Negative results were also obtained when the cotransduction of *xyl* and *livP* was tested. Because there

is a paucity of easily manipulated markers in the *livH--xyl* region (3) and difficulties exist in the genetics of this region (4), precise mapping will require extensive strain constructions.

The linkage of *livP9* with *liv-12* was examined by transduction with P1 phage. The recipient was strain AE410709 (*livH*::Mu *livP9 malT*). Donor phage was grown on strains AE91 (*livH*::Mu *livP*$^+$ *malT*$^+$) and AE125 (*livH*::Mu *liv-12 malT*$^+$). For each donor *malT*$^+$ and *livP*$^+$ (ability to grow on 5 μg on L-leucine per ml) transductants were independently selected; *malT*$^+$ thus served as a control on the efficiency of transduction. In the case where the donor carried *livP*$^+$, the ratio of *malT*$^+$ to *livP*$^+$ transductants was 0.033 (1,100:37). When the donor carried *liv-12*, no *livP*$^+$ transductants were observed (950 *malT*$^+$ transductants scored). We conclude that, because crossover between *liv-12* and *livP9* is rare, these two loci are closely linked. The reason for the low efficiency of transduction of *livP*$^+$ relative to *malT*$^+$ is not apparent; the efficiency was not significantly improved by allowing growth before plating on selective medium. Complementation tests will be required to determine whether *liv-12* and *livP9* are alleles.

DISCUSSION

The mutation *livH* results in the loss of high-affinity uptake of L-leucine through the LIV-I and L-leucine-specific systems in *E. coli* K-12, although the binding proteins are unaltered (1). The residual uptake has been identified as the LIV-II system, by the criteria of ligand specificity (L-leucine, L-valine, and L-isoleucine with K_m's of uptake in the 4- to 7-μM range) and resistance to L-threonine inhibition (2). This system has been characterized previously by ourselves and others (8, 14, 18) in wild-type strains. A system with similar specificity and K_m values, but sensitive to L-threonine inhibition, has been reported by Guardiola and co-workers (9). We have failed to confirm these results, because in all of our mutant strains lacking the high-affinity systems or in wild-type cells in which high-affinity systems are repressed by L-leucine, residual uptake of L-leucine is completely resistant to L-threonine inhibition (2).

Mutant strains selected from a *livH* background (strains AE410709 [*livH livP9*] and AE4107012 [*livH liv-12*]), although selected for the loss of leucine uptake by the LIV-II system, show the expected loss of L-valine and L-isoleucine uptake as well (Table 4, Fig. 1). All other mutant strains from this selection (75 tested) showed a similar phenotype, suggesting that the LIV-II system is a homogeneous carrier for all three amino acids. Although there is a report of specific systems for each amino acid of very high K_m (~200 μM)(8), we would not have detected this type of system at the concentrations of ligand used in these studies. Strain AE4107012 (*liv-12*) shows a minor saturable component for L-leucine of approximately 20 μM K_m without obvious L-valine or L-isoleucine components. We believe that this may represent a K_m defect in the LIV-II system rather than the unmasking of a new system. Genetically, the close linkage of *liv-12* with the null mutation *livP9* supports this hypothesis.

Because the high-affinity binding protein-mediated leucine uptake systems and the LIV-II system operate in the mutational absence of each other (Fig. 3), we infer that they are parallel systems, that is, the leucine-binding proteins probably do not operate by changing the affinity of the LIV-II system as has been suggested for another binding protein transport system, the *mgl* system (15). The data do not exclude the possibility that one or more as-yet-unidentified components are shared. We have, in fact, obtained some indications that a common component may exist for the LIV-I and LIV-II systems. Certain azaleucine-resistant mutants which have not been characterized further were defective in both systems (Rahmanian and Oxender, unpublished data). In addition, the mutation *brnR6*(Am) has also been reported by Guardiola et al. (9) to affect both systems. Another report in the Japanese literature (quoted by Yamato and Anraku [19]) suggests similar mutants. However, of 75 LIV-II mutants selected for this study, all regained LIV-I activity when *livH*$^+$ was introduced by transduction or sexduction (Anderson, unpublished observations), suggesting that pleiotropic mutations in LIV-I and LIV-II must be rare.

Our finding that strains lacking both LIV-I and LIV-II leucine transport systems are apparently unable to retain internal leucine and therefore become weak excretors of L-leucine are similar to results obtained with other transport systems. M. J. Whipp (Ph.D. thesis, University of Melbourne, Australia, 1977) has reported that mutants defective in specific uptake systems for L-tyrosine, L-phenylalanine, and L-tryptophan excrete their respective amino acids, provided that the high-affinity common aromatic transport system (*aroP*) is defective as well. Shifrin et al. (17) have reported histidine excretion in *hisP* mutants, and Rosen (16) has showed excretion of L-arginine in mutants defective in both the lysine-arginine-ornithine system and the arginine-specific system. These common effects can be explained by the inability to retain intra-

cellular amino acids when transport systems are missing. Mutants selected for amino acid analog resistance frequently include transport defectives; if such strains fail to retain their respective metabolites, they might be expected to exhibit derepressed biosynthetic enzyme levels and could be misidentified as regulatory mutants.

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