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UPTAKE AND UTILIZATION OF AROMATIC D-AMINO ACIDS IN *ESCHERICHIA COLI* K12

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SUMMARY

1. The general aromatic amino acid permease system of *Escherichia coli* K-12 efficiently mediates the uptake of the D-isomers of tyrosine and tryptophan. Little or no transport of these D-amino acids occurs in *aroP* mutants, which have lost the general aromatic permease. D-Phenylalanine is transported by this system and by an additional one which does not require the product of the *aroP* gene.

2. Mutations which lead to an ability to utilize D-tryptophan or D-tyrosine do not alter the transport capability with respect to either the relevant D- or L-isomer. Some, but not all, mutations leading to D-tryptophan utilization are accompanied by elevated levels of an aromatic D-amino acid deaminase.

3. Although mutational lesions in the general aromatic permease lead to a loss of ability to utilize D-tryptophan for growth, D-phenylalanine is active as a nutritional supplement in strains lacking the aromatic permease.

4. In *E. coli*, D-tryptophan transport is inhibited by L-tyrosine in two ways: first, through direct competition for a carrier system; second, through repression by L-tyrosine of the general aromatic permease. D-Tryptophan is therefore ineffective as a nutrient in the presence of L-tyrosine, unless cells harbor a newly identified mutation, termed FYO.

INTRODUCTION

For the most part, amino acid requiring mutants of *Escherichia coli* grow only on the L-isomers. In many cases, one can select mutations which permit amino acid auxotrophs to respond to either the D- or the L-isomer [1]. We showed in a previous study that acquisition of the ability to grow on D-tryptophan was the result of a mutation (*dadR*) located at 25.3 min on the *E. coli* chromosome, and that two *dadR* mutants had greatly elevated levels of a D-amino acid deaminase [1].

Mutants capable of utilizing D-tyrosine of D-phenylalanine (phenotypically designated DYU and DFU, respectively) proved to be genetically distinct from *dadR* mutants. We suggested [1] that alterations in transport capability might explain the observed phenotypes.

Each aromatic L-amino acid may enter *E. coli* cells by either of two distinct

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routes [2,3]. A general aromatic permease or one of four highly specific systems functions in the transport of L-histidine, L-phenylalanine, L-tyrosine and L-tryptophan. Ames and Lever [4] demonstrated in *Salmonella typhimurium* that *dhuA* (D-histidine-utilizing) mutants, first reported by Krajewska-Grynkiewicz et al. [5], had gained the ability to transport D-histidine. The new transport activity was mediated by the L-histidine-specific permease system. Secondary mutants, unable to grow on D-histidine, were isolated [4] and some of these lacked certain components of the L-histidine-specific transport system.

It is plausible that D-amino acids may be transported by single permease systems, in contrast to the situation for most L-amino acids, which have multiple transport systems. If this were so, studies of D-amino acid transport would facilitate the investigation of individual permease systems. This line of reasoning predicts that some mutations leading to the loss of ability to use a D-amino acid for growth might come about through loss of transport capability for certain D-amino acids. The present paper shows that these considerations apply in the case of D-tryptophan, which is specifically transported by the general aromatic amino acid permease of *E. coli*.

MATERIALS AND METHODS

Organisms

The various substrains of *E. coli* K-12 employed in the present work are listed in Table I. Most of them are one- or two-step derivatives of the wild-type strains W1485 or Y_{mel} [6]. Transducing phage Plkc is a line that has been carried in our laboratory for 10 years. It was originally obtained from C. Yanofsky.

Strain construction

The *purB* mutation of strain JK248 was isolated in strain JK222 following mutagenesis by irradiation with ultraviolet light (General Electric G15T8 germicidal lamp) to a survival level of 10^{-4} and subsequent penicillin enrichment [7]. Derivatives of JK222 which could no longer utilize D-tryptophan as a tryptophan source were isolated in the same way. The construction of derivative strains by transduction was performed as described by Yanofsky and Lennox [8].

The *trp* deletion associated with the *tonB* locus was introduced into KB3100 by P1 transduction and selection for *tonB* following a period of intermediate growth in broth to permit the expression of resistance to phage T1.

Media and growth conditions

Cells were routinely cultivated in minimal medium E of Vogel and Bonner [9]. D-Glucose (0.2%) was used as the carbon source: for solid media 15 g of Bacto-agar (Difco) and 1 mg of thiamine hydrochloride were added per l. Amino acid growth supplements (20 μg/l) were added as required. For transductional crosses and lysate preparation cells were grown in L broth [10].

Chemicals

D-Tryptophan, D-tyrosine and D-phenylalanine were purchased from Calbiochem; *p*-amino-L-phenylalanine was purchased from Cyclo Chemical. L-[^{14}C]tyrosine,

TABLE I

LIST OF STRAINS

Gene symbols: All gene designations follow those utilized by Taylor and Trotter [22], as follows: *aroB*, dehydroquinate synthetase; *aroP*, general aromatic amino acid transport; *dadR*, D-amino acid deaminase regulation; *leu*, leucine biosynthesis; *purB*, adenylosuccinase; *tonB*, resistance to phage T1; *trp*, tryptophan biosynthesis; *tyrA*, chorismate mutase-prephenate dehydrogenase; *tna*, tryptophanase. The following phenotype designations are used: DYU and DFU: ability to utilize D-tyrosine or D-phenylalanine to satisfy an autoxtrophic requirement for L-tyrosine or L-phenylalanine; FYO: ability to overcome inhibitory effect of phenylalanine and/or tyrosine with respect to utilization of D-tryptophan to satisfy an auxotrophic requirement for L-tryptophan.

Strain	Genotype and/or phenotype
tyr 2–5	*tyrA*
DYU-C	*tyrA* DYU-1
DYU-D	*tyrA* DYU-2
DYU-F	*tyrA* DYU-3
DYU-J	*tyrA* DYU-4
DYU-R	*tyrA* DYU-5
JK202*	prototroph
JK203*	*tyrA*$^-$ DYU-1
JK204*	*tyrA*$^-$ DYU-2
JK205*	*tyrA*$^-$ DYU-3
JK206*	*tyrA*$^-$ DYU-4
KB3100	*aroP*
JK253	*aroP aro*
T3D	*trpE dadR1*
T3A2	*trpE trpA tna*
JK222	*trpE trpA tna dadR1*
JK238	*trpE trpA tna dadR1* FYO
JK248	*trpE trpA tna dadR1 purB*
JK123	*trpE leu dadR1*
JK151	*trpC dadR6*
CT20E	*trpE dadR1*/F′*trp*$^+$
JK157	*trpC dadR6*/F′*trp*$^+$
JK159	*trpC dadR7*/F′*trp*$^+$
JK160	*trpC dadR8*/F′*trp*$^+$
JK246	*tna dadR1* FYO$^+$
JK247	*tna dadR1* FYO
aro 1–4	*aroB*
DFU-A	*aroB* DFU-1
DFU-B	*aroB* DFU-2
JK207*	prototroph
JK208*	*aroB*$^-$ DFU-1
JK209*	*aroB*$^-$ DFU-2
JK100	*tonB trpA–E*▽ *dadR1* FYO$^+$

* Strains JK202–209 are prototrophic transductants isolated following P1 transduction and selection for *tyrA*$^+$ (JK202–206) or *aroB*$^+$ (JK207–209).

L-[^{14}C]tryptophan, D-[^{14}C]tryptophan and D-[^{14}C]tyrosine were obtained from New England Nuclear Corp. All other chemicals were of reagent grade.

Aromatic amino acid uptake

All operations were at 37 °C. Overnight cultures (10 ml) of cells grown in

medium E (or medium E containing L-tyrosine (20 μg/ml) in certain experiments) were centrifuged and the cell pellet resuspended in 1.0 ml of fresh medium E. Half of this suspension was used to inoculate 50 ml of the same medium. After 2 h, when the cultures were in log phase, the cells were harvested by centrifugation and resuspended in approximately 4 ml of minimal medium minus glucose. The turbidity was then adjusted to 200 units, as measured in a Klett-Summerson colorimeter (No. 66 filter) by adding fresh medium. The cells were starved for glucose at least 10 min before conducting uptake assays. The transport capability of our preparations remained constant for at least one hour. The assay for uptake was similar to that employed by Brown [3]. Reaction mixtures contained a ^{14}C-labeled amino acid at an appropriate concentration, non-radioactive inhibitors and 0.2 ml of cell suspension in a total volume of 2.0 ml with final concentrations of salts identical to minimal medium. The experiment was started by addition of the cells. Samples (0.2 ml) were rapidly removed and filtered at various times through nitrocellulose membrane filters (Millipore Corp. HAWP 0.45 μm). The filters were washed free of non-specifically absorbed radioactivity with 3.0 ml of warm (37 °C) minimal medium. The filters were transferred to counting vials, dissolved in 10 ml dioxane-based scintillation fluid [11] and counted in a Beckman cpm-100 scintillation counter.

Brown [3] has shown, and we have confirmed, that cells which have been starved of an energy source retain the ability to actively concentrate aromatic amino acids, although protein synthesis is severely restricted. The deprivation of cells of glucose is technically advantageous because 80–90% of the ingested aromatic amino acids remain in a form which is readily solubilized when the cells are treated with trichloroacetic acid (i.e. very little incorporation into peptide linkage occurs). Because compounds such as 2,4-dinitrophenol and azide inhibit uptake [3] in glucose-starved cells, the transport observed is regarded as active transport.

Enzyme assays

D-Histidine deaminase and D-phenylalanine deaminase were determined as previously described [1]. D-Tyrosine deaminase was measured by following the formation of *p*-hydroxyphenylpyruvate. The reaction mixture contained $5 \cdot 10^{-3}$ M D-tyrosine, 0.1 M sodium phosphate (pH 8.0), and 0.2 ml crude extract in a total volume of 5.0 ml. The conditions for stopping the reaction and chromogen formation were identical to those used in the D-histidine deaminase assay. The absorbancy of *p*-hydroxyphenylpyruvate was measured at 330 nm.

For the assay of transaminase A, reaction mixtures containing L-tyrosine, $5 \cdot 10^{-4}$ M; sodium phosphate 0.05 M (pH 7.6), pyridoxal phosphate $5 \cdot 10^{-5}$ M; sodium α-ketoglutarate $5 \cdot 10^{-3}$ M and crude extract (0.1 ml) were incubated at 37 °C in a total volume of 3 ml. The reaction was terminated by pipetting 1 ml of reaction mixture into 3 ml of NaOH. After 30 min, the concentration of *p*-hydroxyphenylpyruvate was determined spectrophotometrically at 330 nm. Protein concentration was determined by the method of Lowry et al. [12].

RESULTS

Uptake of L-*tyrosine and* D-*tyrosine by wild-type, aroP and DYU strains*

In the DYU strains we have studied, the transport of L-tyrosine and L-trypto-

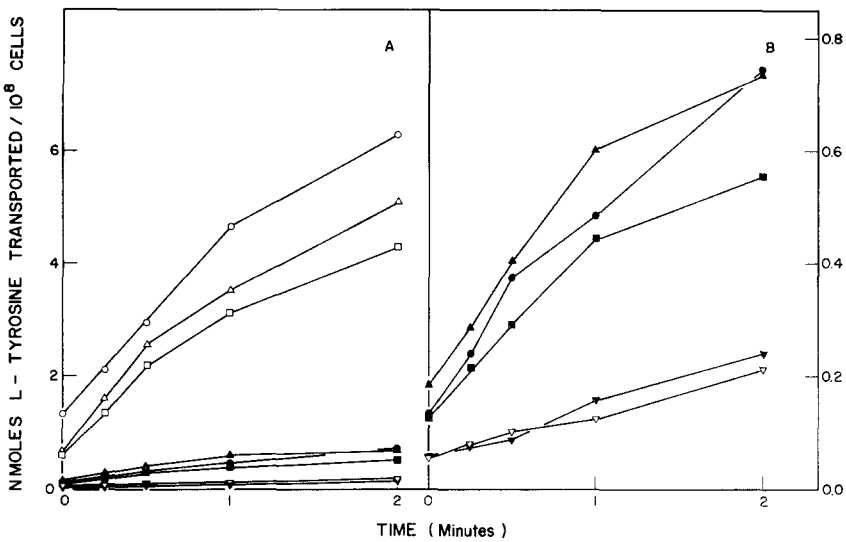

Fig. 1. Transport of L-tyrosine (10^{-5} M) by glucose-starved DYU$^+$ and DYU strains and by an *aroP* mutant. (A) L-Tyrosine uptake mediated by the general aromatic permease was examined for the DYU$^+$ strain JK202 (○), for the DYU strains JK203 (△) and JK204 (□), and for the *aroP* mutant KB3100 (▽). Solid symbols refer to uptake in the presence of 10^{-3} M L-tryptophan. (B) L-Tyrosine uptake mediated by system(s) other than the aromatic permease. Symbols are identical to those in Fig. 1A. Note the difference in scale for Fig. 1B.

phan appeared to be normal. The patterns of uptake in the wild-type strain, JK202, and in two prototrophic strains derived from the D-tyrosine utilizing strains, JK203 and JK204, are indistinguishable from one another and are similar to those reported by Brown [3]. Fig. 1A shows typical data for L-tyrosine transport by the general aromatic permease system and the L-tyrosine specific transport system. L-Tyrosine-specific transport was operationally defined by Brown [3] as transport of L-tyrosine in the presence of excess L-phenylalanine or L-tryptophan.

The data of Fig. 1B also shows that KB3100, an *aroP* mutant, transports L-tyrosine at the same rate in the presence or absence of excess L-tryptophan. This result, which reflects the tyrosine-specific permease, substantiates an earlier finding of Brown [3]. Under our conditions the *aroP*$^+$ strains examined transported about 40% of the available L-tyrosine within 2 min.

The L-tryptophan transport patterns of wild type and the DYU strains, JK203 and JK204, were the same as those found by Brown [3] (data not shown). Mutations to DYU were without effect upon L-tryptophan transport through either the aromatic permease system or the tryptophan-specific transport system. It would therefore appear that the DYU mutation affects neither L-tyrosine nor L-tryptophan transport.

The pattern of D-tyrosine uptake for strain JK202 and the *aroP* mutant KB3100 is shown in Fig. 2. The rate of transport of D-[^{14}C]tyrosine was linear for at least an hour; 2.2% of the D-tyrosine initially present (10^{-4} M) was transported in the first hour. Fig. 2 also shows the effects of L-tryptophan, *p*-amino-L-phenylalanine and L-tyrosine on D-tyrosine transport. When L-tryptophan or *p*-amino-L-phenylalanine was present at a concentration of 10^{-3} M, the transport of D-tyrosine was almost

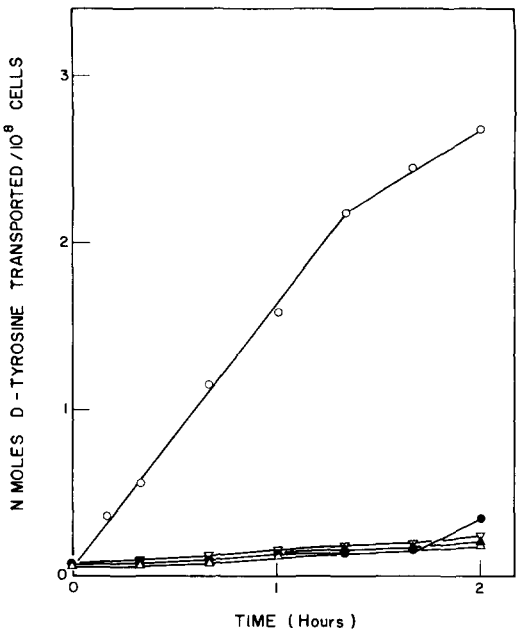

Fig. 2. Transport of D-[^{14}C]tyrosine (10^{-4} M) by JK202 (*aroP*$^+$) in the absence of competing compounds (○) or in the presence of L-tryptophan (10^{-3} M) (△), L-tyrosine (10^{-4} M) (●), or *p*-amino-L-phenylalanine (10^{-3} M) (▲). Transport by KB3100 (*aroP*) of D-[^{14}C]tyrosine was measured in the absence of other aromatic amino acids (▽).

completely abolished. Similarly, L-tyrosine (10^{-4} M) almost completely inhibited D-tyrosine transport. Almost no D-tyrosine transport was observed in KB3100, a strain with a defective aromatic permease. This suggests that the transport of D-tyrosine into *E. coli* requires a functional *aroP* product. A low but clearly significant amount (94 pmoles per 10^8 cells per h) of D-tyrosine was taken up by KB3100. This rate of entry is less than 6% of that which was observed for an uninhibited *aroP*$^+$ strain.

Several strains which contain DYU mutations were examined for D-tyrosine transport. No significant differences between DYU$^+$ and DYU strains were found. Apparently, mutational acquisition of the ability to utilize D-tyrosine is not mediated at the level of transport.

By varying the concentration of D-tyrosine, we estimated that the K_m for D-tyrosine uptake by *aroP*$^+$ cells was $1.5 \cdot 10^{-3}$ M. The V was estimated to be 1.5 nmoles per 10^8 cells per min.

Uptake of D-*tryptophan: role of aroP*

The ability of a wild type strain, JK202, and an *aroP* mutant, KB3100, to concentrate D-tryptophan was studied (Fig. 3). Wild type cells transport D-tryptophan quite efficiently. Approximately 3.3% of the D-tryptophan initially present was concentrated in 4 min. The rate of uptake was linear for the first 2 min, then decreased, probably as a result of the saturation of the internal pool. The presence of a 200-fold excess of unlabeled L-tryptophan, L-tyrosine, L-phenylalanine or *p*-amino-L-phenyl-

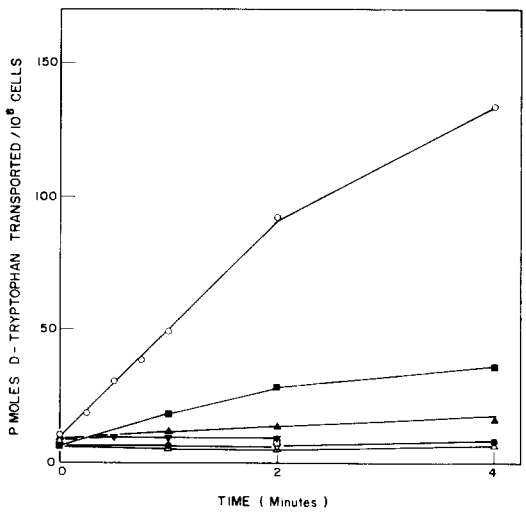

Fig. 3. Transport of D-[^{14}C]tryptophan ($5 \cdot 10^{-6}$ M) by JK202 (*aroP*$^+$) and KB3100 (*aroP*) JK202: no additions (○); L-tryptophan (10^{-3} M) (△); L-tyrosine (10^{-3} M) (□); L-phenylalanine (10^{-3} M) (▽); *p*-amino-L-phenylalanine (10^{-3} M) (●); D-phenylalanine (10^{-3} M) (▲); and D-tyrosine (10^{-3} M) (■). KB3100: (*aroP*). No additions (▼).

alanine abolished the transport of D-[^{14}C]tryptophan (Fig. 3). D-Phenylalanine and D-tyrosine were slightly less potent inhibitors of D-tryptophan transport. These results suggest that D-tryptophan is mostly, if not completely, transported by the aromatic permease system.

The dependence of D-tryptophan transport capability upon the aromatic permease system was supported by studies of an *aroP* mutant. As shown in Fig. 3, KB3100 is incapable of transporting D-tryptophan. If another system besides *aroP* exists which transports D-tryptophan it would have very little transport capability under these conditions.

The kinetic parameters for D-tryptophan transport by JK202 were determined. The K_m was estimated at $5.8 \cdot 10^{-5}$ M and the V as 0.38 nmoles per 10^8 cells per min. Although the V for D-tryptophan transport is only about a fourth of that for D-tyrosine, the aromatic permease system is rapidly transporting D-tryptophan at concentrations well below those where D-tyrosine transport is detectable. An equivalent amount of either D- or L-tryptophan per cell will satisfy the growth requirements of strains of *E. coli* that are *trp dadR*. In addition the growth rate of many *trp dadR* strains is the same with either D- or L-tryptophan as a supplement. A simple calculation shows that the total amount of D-tryptophan required ($1 \cdot 10^{-17}$ moles) per cell can be transported in about 4 min under the growth conditions (20 μg/ml D-tryptophan) employed in our studies. Since the generation time in minimal medium is about 70 min the rate of D-tryptophan transport is more than adequate to maintain this growth rate. It takes 9.5 min to transport an equivalent amount of D-tyrosine under these conditions.

Genetic analysis of D-*tryptophan transport*

Since strains of *E. coli* exist which can use either D- or L-tryptophan to satisfy

their auxotrophic requirement, a direct test of whether all D-tryptophan is transported by the aromatic permease system was possible. Three separate lines of evidence showed that, in vivo, all D-tryptophan is transported by the aromatic permease system. First, when JK223 (*aroP trp▽A-E purB*) was transduced by Plkc phage grown on JK222 (*trpA trpE dadR*), with selection for *purB*$^+$, none of the transductants could grow on D-tryptophan although 50% would be expected to do so [1]. No transductants were found when selection was for *dadR*. The presence of *aroP* in the recipient evidently prevents the expression of the *dadR* phenotype. Secondly, with JK123 (*trpE dadR leu aroP*$^+$) as a recipient and KB3100 (*trp*$^+$ *dadR*$^+$ *leu*$^+$ *aroP*) as a donor in a transduction cross, and selection for *leu*$^+$, 6 out of 56 colonies (11%) failed to grow on D-tryptophan. About 30% of the *leu*$^+$ transductants should be *aroP* since *leu* and *aroP* are closely linked [3], while none should be *dadR*$^+$ or *trp*$^+$. The co-segregation of *aroP* with the inability to utilize D-tryptophan provides very strong evidence that an *aroP*$^+$ gene is necessary for the growth of *trp dadR* strains on D-tryptophan. A third piece of evidence for the role of *aroP* in D-tryptophan utilization was provided by the isolation, following mutagenesis and penicillin selection, from a *trp dadR* parent strain, JK222, of mutants that could no longer use D-tryptophan. Many such mutants were obtained. From each of ten independent tubes, two mutants were analyzed for linkage between the mutation preventing D-tryptophan utilization and *leu*. Transductional analysis showed that all the mutations were linked to *leu*. Most of these mutations exhibited no leakness and only low reversion rates (approx. $1 \cdot 10^{-8}$). Such mutations therefore probably represent lesions in the *aroP* gene. Therefore, it seems clear that loss of the general aromatic permease prevents the utilization of D-tryptophan by *dadR* strains. It follows that if *E. coli* cells possess other systems capable of mediating D-tryptophan uptake, they are insufficient to allow the D-isomer to function nutritionally.

Genetic evidence that D-*phenylalanine is transported by a system in addition to the aromatic permease*

D-Phenylalanine is capable of inhibiting D-tryptophan transport (Fig. 3). Since all D-tryptophan is apparently transported via the aromatic permease, it seems probable that D-phenylalanine can also be transported by this system. Many aromatic metabolites as well as their analogues have been found to be transported by this system [2]. In addition D-phenylalanine can inhibit L-phenylalanine transport [13].

To test the possibility that all D-phenylalanine is transported by the aromatic permease system, experiments analogous to those in the preceding section were performed. Strain JK253 (*aro aroP*) was used as a recipient in a cross where the donor Plkc phage came from strain DFU-B (*aroB DFU*), a strain harboring a mutation (DFU-2) allowing the growth of an *aro* mutant on either D- or L-phenylalanine in the presence of L-tryptophan, L-tyrosine, *p*-aminobenzoic acid and *p*-hydroxybenzoic acid. Selection was for growth on D-phenylalanine in the presence of the other aromatic supplements. Two kinds of transductants were found; first, *aro*$^+$, representing replacement of the *aro* mutation (the induced *aro* mutation was probably not *aroB*), and second, DFU strains that were still *aro* (approx. 10%) but which grew on D- as well as L-phenylalanine. Since the second class is the triple mutant *aro aroP DFU*, the presence of a mutation in *aroP* does not block D-phenylalanine utilization. Therefore *E. coli* possesses a second transport system for D-phenylalanine which can

mediate the uptake of this amino acid at a rate sufficient for supporting growth.

Enzyme analysis of DYU, DFU and dadR strains

In light of the finding [1] that some *dadR* strains contained very high levels of an enzyme capable of deaminating D-phenylalanine and D-histidine, a partial analysis of certain enzymes was carried out on extracts of *dadR*, DYU and DFU mutants. Most strains were transduced to prototrophy before growth to avoid the possible complication of repression of the relevant enzymes by growth supplements. Certain strains were examined without transduction to prototrophy. The results of these experiments are given in Table II.

Our DYU strains consist of two classes. The first class, which includes DYU-D and DYU-F, grow slowly on minimal medium and are strongly stimulated by D-tyrosine. As shown in Table II, these two strains yield extracts which contain a slightly higher level of D-amino acid deaminase than the parental strain or strains from the second class, which exhibit no leakiness on minimal medium. These strains are represented by DYU-C, DYU-J, and DYU-R. Growth of DYU-J and DYU-R with D-tyrosine as a tyrosine source did not lead to higher levels than those found in prototrophic derivative strains of the same class. Neither DYU class had unusual levels of transaminase A [14] which catalyzes the terminal transamination step in L-tyrosine biosynthesis.

TABLE II

ENZYME ACTIVITIES OF DYU$^+$, DYU, DFU$^+$, *dadR*$^+$ AND *dadR* STRAINS

The preparation of extracts and enzymatic assays are given in Materials and Methods. The activities are as follows: D-histidine as substrate, ΔA/mg protein in 30 min; D-phenylalanine as substrate, ΔA/mg protein in 30 min; D-tyrosine as substrate, ΔA/mg protein in 6 min; transaminase A, nmoles/mg protein per min. Supplements were present at 20 μg/ml.

Strain	Phenotype or genotype	Supplement	Deamination			Transaminatio
			D-Histidine	D-Phenyl-alanine	D-Tyrosine	L-tyrosin
JK202	DYU$^+$		0.026		0.083	41.1
JK203	DYU-1		0.023		0.082	38.7
JK204	DYU-2		0.169		0.484	49.2
JK204	DYU-3		0.122		0.282	41.4
JK206	DYU-4		0.026		0.060	41.7
tyr 2–5	tyrA	L-Tyrosine	0.049		0.059	20.2
DYU-J	tyrA DYU-4	L-Tyrosine	0.043		0.080	33.3
DYU-R	tyrA DYU-5	L-Tyrosine	0.087		0.288	43.2
JK207	DFU$^+$		0.025	0.216	0.081	
JK208	DFU-1		0.032	0.637	0.201	
JK209	DFU-2		0.035	0.536	0.170	
JK151	dad R1		1.56	16.0		*
T3D	trpE dad R1	D-Tryptophan	0.873	6.43	6.17	
JK157	dad R6		0.028	0.168	0.374	
CT20E	trpC dad R6	D-Tryptophan	0.026		0.183	
JK159	*trpC dadR7*/F'trp$^+$		0.015	0.055	0.025	
JK160	*trpC dadR8*/F'trp$^+$		0.920	6.48	7.67	

* The transaminase A level in a different strain, dadR1, was 48.9.

None of the DFU mutant strains are able to grow on minimal medium. In addition they display no increased capability to deaminate D-amino acids (Table II, lines 9–11). The mechanism of D-tyrosine utilization by non-leaky DYU strains and of D-phenylalanine by DFU strains does not seem to involve changes in D-amino acid deamination ability.

Although we reported [1] that *dadR* strains contain high levels of a D-amino acid deaminase, the data of Table II show that some strains contain only normal, parental levels. Of sixteen strains examined, seven contain 5–30 times as much enzyme while the other nine do not differ significantly from their parents. By chance, the two strains characterized previously were in the group containing high levels of the enzyme. When the ability to utilize D-tryptophan in a strain with high levels of D-amino acid deaminase was transferred by Plkc transduction to another strain the high enzyme levels were invariably cotransduced with the ability to use D-tryptophan. It appears that the mutants previously designated as *dadR* are actually divisible into two classes on the basis of differences in D-amino acid deaminase levels, although all D-tryptophan utilizing strains we have found show equal linkage to both *purB* and the *trp* operon. Further study will be required to more adequately characterize this family of mutants.

Isolation and characterization of FYO mutants

Because all D-tryptophan is transported by the aromatic permease system, other compounds transported by this system competitively inhibit D-tryptophan utilization. Strain JK222, (*trpE trpA dadR tna*) was chosen as a parental strain for experiments employing inhibitors of D-tryptophan transport. L-Tyrosine and L-phenylalanine were both found to inhibit the growth of JK222 when D-tryptophan was supplied as a tryptophan source. This strain cannot grow on minimal plates containing D-tryptophan (20 μg/ml) and L-tyrosine or L-phenylalanine (20 μg/ml). Nineteen mutants were isolated which could grow on D-tryptophan in the presence of L-tyrosine or L-phenylalanine. These mutants were of spontaneous origin or induced with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, ethylmethane sulfonate or ultraviolet light. Individual growth rates on this medium varied widely. All strains selected for resistance to L-phenylalanine were also resistant to L-tyrosine and vice versa. A fast growing strain, JK238, carrying a mutation induced by ultraviolet irradiation, was chosen for further study. The symbol FYO was selected for the mutant phenotype (F, phenylalanine; Y, tyrosine; O, overcomes) and FYO^+ for wild type sensitivity.

When D-tryptophan is the source of the required tryptophan, L-tyrosine and L-phenylalanine would be expected to inhibit the growth of *trp dadR* strains by interfering with the transport of this compound. At least three theories could explain the mechanism of resistance of FYO mutations: the appearance of a separate transport system for D-tryptophan, not inhibited by L-tyrosine or L-phenylalanine, resistance of the aromatic permease system itself to L-tyrosine or L-phenylalanine, or constitutive production of the aromatic permease system. The first two hypotheses would predict that FYO cells grown on minimal medium should show higher levels of transport of D-tryptophan in the presence of L-tyrosine or L-phenylalanine than FYO^+ cells under the same conditions. Neither of these explanations for resistance seems to be correct (Fig. 4). No major differences between D-trytophan transport capability in FYO or FYO^+ strains appeared when either L-phenylalanine or L-tyrosine was present at concentrations equal to that of D-tryptophan.

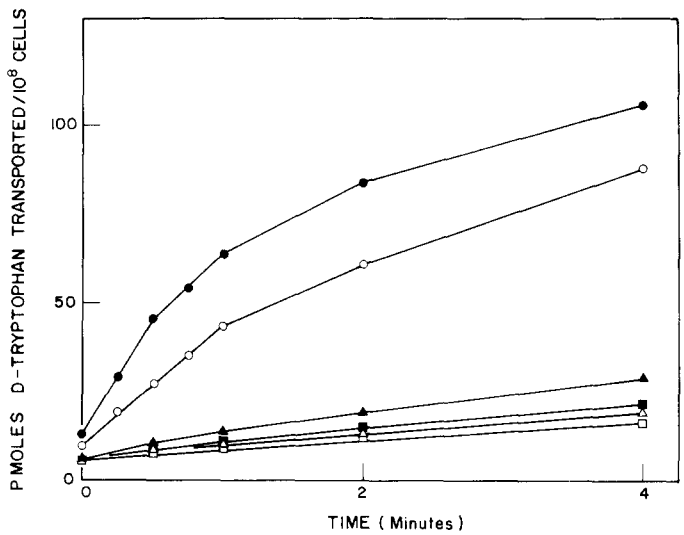

Fig. 4. D-Tryptophan transport in FYO⁻ and FYO strains. All aromatic amino acids were present at final concentrations of $5 \cdot 10^{-6}$ M. Transport of D-[^{14}C]tryptophan by JK246 (FYO$^+$): no additions (○); L-tyrosine (△); L-phenylalanine (□). Transport of D-[^{14}C]tryptophan by JK247 FYO): no additions (●); L-tyrosine (▲); L-phenylalanine (■).

If FYO cells grown in the presence of L-tyrosine or L-phenylalanine showed a much higher transport activity for D-tryptophan than FYO$^+$ cells, the third possibility would be favored. This also is apparently not the case. As Fig. 5 shows, FYO and FYO$^+$ strains have similar levels of D-tryptophan transport after growth in L-tyrosine. However, L-tyrosine apparently does repress the aromatic permease, since the level of D-tryptophan transport has been greatly lowered (compare Figs. 3 and 4 to Fig. 5). The activity that does remain is inhibited by the presence of L-tyrosine in the transport assay to the same degree in both strains.

The FYO phenotype is weakly cotransducible with *trp*. When appropriately marked strains are used, one can obtain positive selection for FYO on plates containing D-tryptophan and L-phenylalanine (20 μg/ml). In one experiment, a P1 transduction cross was performed using JK 100 (*trpγA-E dadRl FYO*$^+$) as recipient and JK238 (*trpE trpA dadRl FYO*) as a donor. Selection for growth on indole resulted in transductants which had incorporated *trpA* and/or *trpE*. Among 57 transductants capable of growth on either indole or tryptophan, two had acquired the FYO phenotype. This degree of linkage is about the same as that for *trp* and *dadR*. However, it has not been established on which side of the *trp* operon the FYO locus is located.

Repression of the aromatic and tyrosine-specific transport systems by L-*tyrosine*

The observation that L-tyrosine represses D-tryptophan transport (Fig. 5) led to further experiments on the repression by L-tyrosine of the transport systems we have been studying. The L-tyrosine specific transport system was found to be strongly repressed by growth with L-tyrosine (20 μg/mg) (Fig. 6A). The transport of L-[^{14}C]-tyrosine by the aromatic permease system (gross transport of L-[^{14}C]tyrosine minus specific transport) was also repressed by L-tyrosine (Fig. 6B).

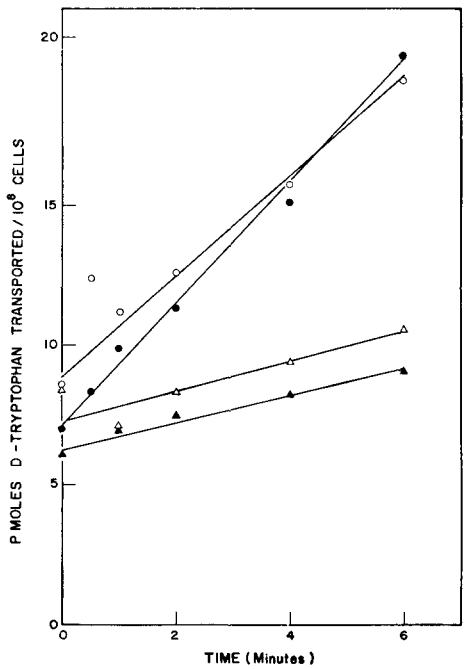

Fig. 5. Aromatic permease levels in FYO$^+$ and FYO strains by JK246 (FYO$^+$) and JK247 (FYO) after growth in the presence of L-tyrosine (20 μg/ml): JK246 (○); JK247 (●). Transport by the same cells with L-tyrosine ($5 \cdot 10^{-6}$ M) included in the uptake assays: JK246 (△); JK247 (▲).

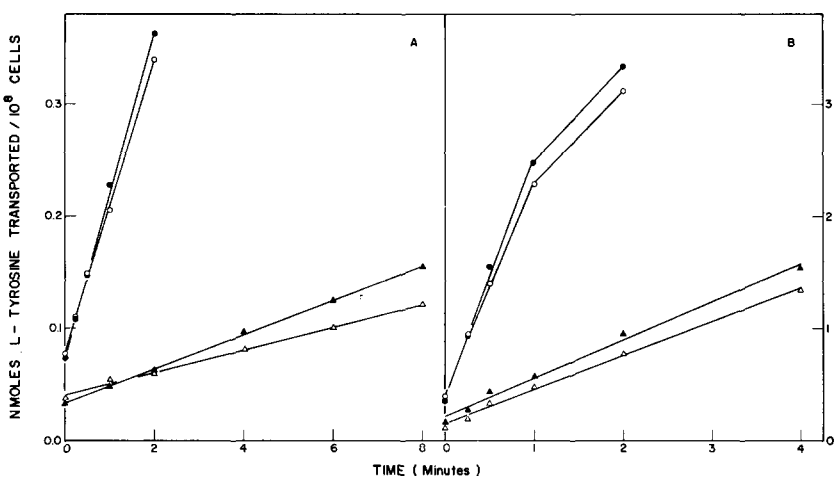

Fig. 6. The influence of growth in the presence of L-tyrosine on L-tyrosine-specific transport and general aromatic transport. (A) Specific uptake of L-[^{14}C]tyrosine (10^{-5} M) in the presence of L-tryptophan (10^{-3} M); JK246 (○) and JK247 (●) grown without added L-tyrosine; JK246 (▲) and JK247 (△) grown with added L-tyrosine (20 μg/ml). (B) General uptake of L-[^{14}C]tyrosine (10^{-5} M): JK246 (○) and JK247 (●) grown without added L-tyrosine; JK246 (▲) and JK247 (△) grown with added tyrosine (20 μg/ml).

TABLE III

TRANSPORT OF L-TYROSINE AND D-TRYPTOPHAN BY JK246 AND JK247 AFTER GROWTH WITH OR WITHOUT L-TYROSINE

Transport of L-[^{14}C]tyrosine as measured under the conditions of Fig. 6 or D-[^{14}C]tryptophan as in Fig. 3. L-Tyrosine transport is in nmoles/10^8 cells per min and D-tryptophan in pmoles/10^8 cells per min. Relative transport is based on the rate of the tyrosine-grown cells.

Transport	JK246 (FYO$^+$)		JK247 (FYO)	
	min* (nmoles)	min + L-tyrosine** (nmoles)	min* (nmoles)	min + L-tyrosine** (nmoles)
L-[^{14}C]Tyrosine				
Gross transport	1.90	0.306	2.13	0.339
Aromatic transport	1.77	0.296	1.99	0.324
Relative aromatic transport	5.97	1.00	6.13	1.00
Tyrosine specific transport	0.130	0.0101	0.145	0.0154
Relative specific transport	12.9	1.0	9.41	1.00
D-[^{14}C]Tryptophan	(pmoles)	(pmoles)	(pmoles)	(pmoles)
Aromatic transport	33.5	1.45	66.0	1.99
Relative aromatic transport	23.1	1.0	33.1	1.00
Inhibited with L-tyrosine	3.13	0.464	5.82	0.471
% activity remaining	9.36	32.1	8.82	23.6

* Minimal medium.

** Minimal medium containing L-tyrosine (20 μg/ml).

The initial rates of transport for D-tryptophan and L-tyrosine through the aromatic permease system as well as L-tyrosine transport through the tyrosine specific transport system were calculated for growth with and without L-tyrosine. The data for strains JK246 (*dadRI FYO*$^+$) and JK247 (*dadRI FYO*), summarized in Table III, indicated that L-tyrosine represses L-tyrosine-specific transport by about 10-fold. L-[^{14}C]Tyrosine transport through the aromatic permease system is repressed about 6-fold, whereas roughly a 25-fold repression of D-tryptophan transport is observed.

DISCUSSION

Our results suggest that studying D-amino acid uptake is a useful method for focusing upon individual transport systems. All D-tryptophan and most, if not all, D-tyrosine are transported by the aromatic permease system. This fact simplifies a concerted biochemical and genetical approach to the study of transport in this system. Genetic techniques frequently cannot be applied to L-amino acid transport studies because these compounds have a number of independent routes for uptake.

A number of mutations in transport systems have been described [15]. Mutations in the *aroP* gene have previously been isolated by selection for resistance to azaserine [2] or 5-methyltryptophan, fluorophenylalanine and thienylalanine [3]. The great majority of the mutants resistant to analogs were not *aroP*. In contrast, the selection employed here led to the facile isolation of *aroP* strains (based on linkage to *leu*). Reversion of such mutations was detected by simply plating on selective (D-tryptophan-containing) medium.

The biochemical route followed by D-tryptophan in its utilization by *dadR* strains is presently unclear. Perhaps there are two routes, only one of which involves deamination. Possibly the high levels of D-amino acid deaminase in half the *dadR* strains are an indirect result of a genetic change essential to the process of D- tryptophan utilization. The utilization of D-tyrosine by DYU strains and D-phenylalanine by DFU strains seems not to involve major changes in the transport or deamination systems for these D-amino acids. Nor is L-tyrosine transaminase affected by mutation to DYU. In view of the findings that D-tyrosine is activated and used in vitro for protein synthesis [16] and that D-tyrosine can replace L-tyrosine in vivo [17], further biochemical investigations of the protein synthesizing apparatus with respect to D-tyrosine utilization are indicated. The tyrosine tRNA deacylase [18] as well as racemization after activation [19] must also be explored as a possibility.

The repression of an amino acid transport system by growth on that amino acid has been reported for leucine [20,21] in *E. coli*. Similarly, L-tyrosine repressed the formation of the aromatic permease and the L-tyrosine specific transport systems. However, the apparent degree of repression of the aromatic permease system varied, depending on how the activity of the system was measured. There was a greater reduction of D-tryptophan transport capability than of L-tyrosine. Selection of FYO mutations, which were expected to change the sensitivity of the aromatic permease system to repression by L-tyrosine or to inhibition of D-tryptophan transport by L-tyrosine, did not seem to affect this system in one mutant examined. Only further investigations will reveal whether some of these mutations affect properties of the transport system.

The difference in the extent of repression of the aromatic permease system as measured by L-tyrosine uptake or D-tryptophan uptake may mean that non-identical routes are followed by these substances during uptake, or that tyrosine-grown cells contain a pool of sufficient size to inhibit D-tryptophan uptake. The former explanation would hold if there were a 25-fold repression of a component normally limiting for D-tryptophan transport but non-limiting for L-tyrosine transport. After repression the component would become limiting for L-tyrosine transport and lead to a decrease in transport. The second explanation postulates a 6-fold repression of the aromatic permease system together with the retention of a sizable L-tyrosine pool after growth with L-tyrosine. The rate of tyrosine transport would only reflect the lowered level of permease since the rate is usually independent of the presence of a pool because the exchange reaction proceeds at the same rate as the initial rate in cells grown without L-tyrosine. However, the exit and recapture reactions with L-tyrosine could significantly decrease the rate of transport for D-tryptophan. Another feature of the data (Table III) is that the residual transport of D-tryptophan after repression by L-tyrosine is about 3 times as resistant to L-tyrosine as is the non-repressed transport.

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REFERENCES

1 Kuhn, J. and Somerville, R. L. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2484–2487
2 Ames, G. F. (1964) Arch. Biochem. Biophys. 104, 1–18
3 Brown, K. D. (1970) J. Bacteriol. 104, 177–188
4 Ames, G. F. and Lever, J. (1970) Proc. Natl. Acad. Sci. U.S. 66, 1096–1103
5 Krajewska-Grynkiewicz, K., Walczak, W. and Klopotowski, T. (1971) J. Bacteriol. 105, 28–37
6 Bachmann, B. J. (1972) Bacteriol. Rev. 36, 525–557
7 Adelberg, E. A. and Myers, J. W. (1953) J. Bacteriol. 65, 348–353
8 Yanofsky, C. and Lennox, E. S. (1959) Virology 8, 425–447
9 Vogel, H. J. and Bonner, D. M. (1956) J. Biol. Chem. 218, 97–106
10 Lennox, E. S. (1955) Virology 1, 190–206
11 Miller, D. L. and Rodwell, V. W. (1971) J. Biol. Chem. 246, 1765–1771
12 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
13 Piperno, J. R. and Oxender, D. L. (1968) J. Biol. Chem. 243, 5914–5920
14 Rudman, D. and Meister, A. (1953) J. Biol. Chem. 200, 591–604
15 Lin, E. C. C. (1970) Annu. Rev. Genet. 4, 225–262
16 Calendar, R. and Berg, P. (1966) Biochemistry 5, 1690–1695
17 Champney, W. S. and Jensen, R. A. (1970) J. Bacteriol. 104, 107–116
18 Calendar, R. and Berg, P. (1967) J. Mol. Biol. 26, 39–54
19 Kurahashi, K., Yomada, M., Mori, K., Fujikawa, K., Kambe, M., Imae, Y., Sato, E., Takahashi, H. and Sakamoto, Y. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 815–826
20 Inui, Y. and Akedo, H. (1965) Biochim. Biophys. Acta 94, 143–152
21 Penrose, W. R., Nichoalds, G. E., Piperno, J. R. and Oxender, D. L. (1968) J. Biol. Chem. 243, 5921–5928
22 Taylor, A. L. and Trotter, C. D. (1972) Bacteriol. Rev. 36, 504–524