A MUTANT CONSTITUTIVE FOR AROMATIC PERMEASE

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1. Introduction

The aromatic amino acids are transported in *Escherichia coli* by a combination of the general aromatic permease and permeases specific for each amino acid [1,2]. In contrast, D-tryptophan enters the cell only via the aromatic permease [3]. Mutants lacking this permease have been isolated [2,3]. The aromatic permease is repressed by L-tyrosine [3].

Since *trp dadR* strains growing on D-tryptophan are absolutely dependent on aromatic permease activity, an attempt was made to isolate constitutive mutants on the basis of L-tyrosine resistance [3]. However the major class obtained involved changes in D-tryptophan oxidase [4] which like the aromatic permease is sensitive to competitive inhibition by L-tyrosine. We therefore sought compounds that are without effect on the oxidase. By using resistance to 5-methyltryptophan we were able to isolate the desired constitutive mutant.

2. Materials and methods

2.1. *Bacterial strains*

JK222 *trpA trpE dadR tna*, JK246 *trp*$^+$ *dadR tna*, W3110 wild type.

2.2. *Medium*

Medium E of Vogel and Bonner [5] containing 0.2% glucose, D-tryptophan, L-tyrosine, 5-methyl-DL-tryptophan and 5-fluoro-DL-tryptophan added as indicated at 20 μg/ml.

2.3. *Transduction*

The procedure of Yanofsky and Lennox [6] with bacteriophage Plkc was used.

2.4. *Uptake experiments*

Concentrated log-phase cells adjusted to 200 Klett units (No. 54 filter) and starved for glucose in Medium E were diluted into the same medium containing the ^{14}C-labeled amino acid as previously described [3]. Samples were removed, filtered through Millipore HAWP filters, washed thoroughly and counted.

2.5. *Radioactive compounds*

L-[^{14}C]Tyrosine and D-[^{14}C]tryptophan were purchased from New England Nuclear Corp.

3. Results and discussion

3.1. *Mutant isolation*

Both 5-methyl-DL-tryptophan (5MT) and 5-fluoro-DL-tryptophan (5FT) effectively block D-tryptophan utilization by *trp dadR* strains. Since they do not strongly inhibit D-tryptophan oxidase and enter the cell via the aromatic permease, there is a strong possibility that resistant mutants will be affected in the latter step. Resistant mutants were isolated by plating JK222 on minimal agar plates containing D-tryptophan and 5MT or 5FT. Mutants resistant to 5MT were easily isolated while those resistant to 5FT were rare. This difference is probably due to the near inability of 5MT to be attached to tryptophanyl-tRNA [7] while 5FT is known to enter proteins in place of tryptophan [8]. The mutants obtained as well as those previously isolated as L-tyrosine and L-phenylalanine resistant [3] that did not represent changes in D-tryptophan oxidase (FYO-II) were examined for L-tyrosine and D-tryptophan transport.

3.2. *Uptake studies*

After transduction to *trp*$^+$ every strain was examined with respect to uptake via the aromatic permease. In one set of experiments, cells for uptake were obtained from cultures to which no L-tyrosine had been added; in a second set the culture medium contained L-tyrosine (20 μg/ml). The uptake of L-[^{14}C]tyrosine in the presence or absence of an inhibiting concentration of unlabeled D-tryptophan and that of D-[^{14}C]tryptophan with or without an inhibiting concentration of cold L-tyrosine were determined. Neither five FYO-II strains nor one that was resistant to 5FT differed significantly from their parent strain in these experiments. Among the 6 strains that were resistant to 5MT, 3 had altered properties. Two of them had slower rates of D-[^{14}C]tryptophan uptake but growth with L-tyrosine did not repress their D-tryptophan uptake. A third strain had a more dramatic phenotype of the same kind.

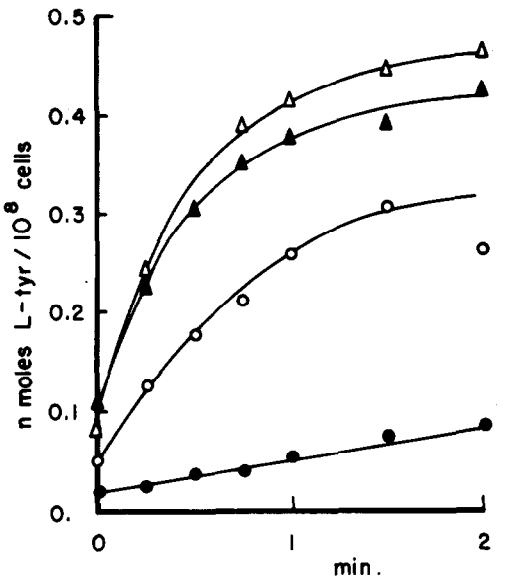

Fig.1. Uptake of L-[^{14}C]tyrosine as a function of time. Strain JK246 (*trp*$^+$ *dadR*) and its mutant derivative HH7 (*trp*$^+$ *dadR*, resistant to 5MT) were assayed for active transport of L-tyrosine as described in Materials and methods. The abscissa is time in minutes. The uptake mixture contained L-tyrosine at a final concentration of 1 μM. JK246 grown in minimal medium (○), JK246 grown in medium containing L-tyrosine (●), HH7 grown in minimal medium (△) or with L-tyrosine (▲). The ordinate is 10-fold less than reported previously [3] because of a mistake on the part of the supplier.

The L-[^{14}C]tyrosine uptake profile of this mutant and that of its parent are shown in fig.1. Not only does the mutant exhibit more rapid uptake but in contrast to its parent this uptake was not repressed by growth with L-tyrosine. This effect does not result from differences in the internal pool of L-tyrosine since the uptake (exchange) rate in cells preloaded with unlabeled L-tyrosine is identical to that without preloading. Addition of chloramphenical (100 μg/ml) had no effect. As shown in table 1, the inhibition caused by adding cold D-tryptophan is normal.

Concomitantly, the rate of uptake of D-[^{14}C]tryptophan is greater in the mutant and this uptake is also not repressible by L-tyrosine (fig.2). This uptake is inhibited to the same extent as that of its parents by the addition of cold L-tyrosine to the uptake reaction mixture (table 2). Thus the difference between these strains is their repressibility by L-tyrosine rather than a change in the properties of the aromatic permease itself.

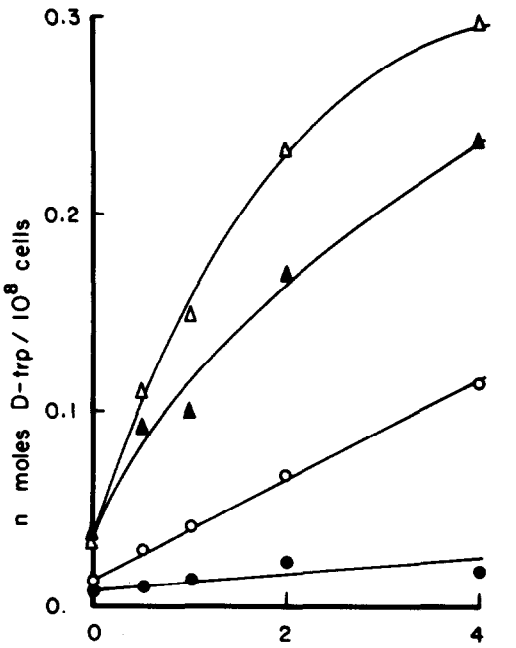

Fig.2. Uptake of D-[^{14}C]tryptophan as a function of time. The two strains of fig.1 above were analysed for active transport of D-tryptophan which was present at a final concentration of 5 μM. JK246 grown in minimal (○), grown with L-tyrosine (●), HH7 grown in minimal (△), with L-tyrosine (▲).

Table 1
Initial rates of uptake

Uptake	Strain	Growth medium	Inhibitor	pmol/min/10^8 cells	% Activity remaining
D-[^{14}C]Tryptophan	JK246	minimal	–	28.1	
		minimal	L-tyr	2.75	9.8
		+L-tyr	–	8.85	
		+L-tyr	L-tyr	1.24	14
	HH7	minimal	–	97.4	
		minimal	L-tyr	11.9	12
		+L-tyr	–	109	
		+L-tyr	L-tyr	9.8	9.0
L-[^{14}C]Tyrosine	JK246	minimal	–	206	
		minimal	D-trp	66.2	32
		+L-tyr	–	33.0	
		+L-tyr	D-trp	18.5	56
	HH7	minimal	–	331	
		minimal	D-trp	83.8	25
		+L-tyr	–	266	
		+L-tyr	D-trp	70.4	26

The two strains of fig.1 were analyzed for uptake of D-tryptophan (5 μM) in the presence or absence of L-tyrosine (5 μM) after growth in minimal medium or minimal medium containing L-tyrosine (20 μg/ml). L-Tyrosine uptake was analyzed in the same way except that L-tyrosine (L-tyr) was present at 1 μM and the unlabeled inhibitor, D-tryptophan (D-trp), when present was at a final concentration of 1 mM.

To determine whether D-tryptophan is metabolized during uptake, cells were collected by filtering after a 6 min period of uptake and boiled to extract small molecules. Centrifuged extract was taken to dryness and resuspended in a small volume of water. There was essentially no loss of radioactivity during this procedure. A 10 μl portion of the extract was chromatographed with cold D-tryptophan as a carrier on Merck thin-layer silica-gel (catalog number 5748) using *n*-propanol, 2-propanol, water and ammonia in the ratio of 4:3:2:1. Of the recoverable counts (~30%), 93% of the radioactivity co-chromatographed with D-tryptophan (R_F = 66).

3.3. *Other properties of the constitutive mutant*

Because a previous study had revealed that resistance to L-tyrosine by *dadR* strains utilizing D-tryptophan results from changes in D-tryptophan oxidase, the level of enzyme and its properties were compared in the mutant and its parent. No differences were found in either the amount of enzyme as judged by specific activity or in its susceptibility to L-tyrosine inhibition.

The untransduced mutant strain and its parent (JK222) were used as donors in a transductional cross with an *aroP* strain of appropriate genotype. After selection for the ability to utilize D-tryptophan (*aroP*$^+$) the transductants were checked for resistance to 5MT on D-tryptophan containing plates and for their *trp* phenotype. All were *trp*. With the constitutive mutant as a donor all transductants were resistant to 5MT; with its parent all were sensitive. Thus the mutation causing constitutivity must lie very close to the *aroP* locus. Since dominance tests have not been performed we do not yet know whether the mutation represents a change in an operator or a repressor gene.

Acknowledgement

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