Effects of *aroP*$^-$ Mutation on the Tryptophan Excretion in *Escherichia coli*

Chi, Youn-Tae, Byung-Woo Ahn and Se-Yong Lee

Department of Agricultural Chemistry, College of Agriculture, Korea University, Seoul 132, Korea

aroP$^-$ 변이가 *E. coli*에서 트립토판 방출에 미치는 영향

지연태 · 안병우 · 이세영

(고려대학교 농과대학 농화학과)

As a part of the host cell development for a amplified recombinant *trp* operon, *aroP*$^-$ mutation was introduced in a *E. coli* host strain. *aroP*$^-$ mutation was induced by transposon Tn*10* and transduced into the *E. coli* host cell by bacteriophage P1*Kc*. The effect of *aroP*$^-$ mutation on the excretion of tryptophan in *E. coli trpR*$^{-ts}$/ColE$_1$-trp$^+$ cells was investigated. Mutant lacking the general aromatic transport system was resistant to β-2-thienylalanine (2×10^{-4} M), p-fluorophenylalanine (2×10^{-4} M), or 5-methyltryptophan (2×10^{-4} M.) [^{3}H]-tryptophan uptake of the *aroP*$^-$ mutant strain was reduced considerably as compared with *aroP*$^+$ counterpart. The rate of [^{3}H]-tryptophan uptake of the *aroP*$^-$ mutant strain treated with NaN_3 (3×10^{-2} M) was much less affected than that of *aroP*$^+$ counterpart. The *aroP*$^-$ transductants increased the tryptophan excretion from *E. coli trpR*$^{-ts}$/ColE$_1$*trp*$^+$ four times more than *aroP*$^+$ counterpart.

The aromatic amion acids, tryptophan, tyrosine, and phenylalanine, are taken up in *Escherichia coli* K-12 and *Salmonella typhimurium* by two transport systems (Brown, 1970). First, a general (common) transport system, specified by the gene *aroP*, transports all three aromatic amino acids. The second system is specific for each aromatic amino acid, either tryptophan, tyrosine, or phenylalanine (Whipp *et al.*, 1977, Whipp *et al.*, 1980). In addition to the above two systems, there is also an inducible system for transport of tryptophan. This system is subject to catabolite repression and is not induced in the presence of glucose.

It has previously been shown that cells grown in the presence of aromatic amino acid have decreased levels of the general transport system and the specific transport system (Kuhn *et al.*, 1974 Thorne *et al.*, 1975). The present paper describes a mutant lacking the general transport system (*aroP*$^-$) and effect of the *aroP*$^-$ mutation on the excretion of trytophan in *E. coli trpR*$^{-ts}$/ColE$_1$-*trp*$^+$.

MATERIALS AND METHODS

Organisms: The strains of *E. coli* K-12 and phages used are listed in Table 1.

Media and growth conditions: Cells were grown on minimal medium as described by Vogel-Bonner (1956) or in L broth of Luria and Burrous (1957) with shaking at 37 °C on gyrotary shakers. Growth was measured as the increase in absorbancy at 580 nm by use of a 1-cm curette and a Hitachi spectrophotometer.

Chemicals: Inorganic chemicals were of analytical

Table 1. Strains and phages used

Strains/Phages	Relevant properties	Source
E. coli K-12		
W3110	Wild type	C. Yanofsky
HB101	*leu thr thi hsdM hsdR*	H. S. Kang
KUB39	$trpR^{-ts}$ *tna* $trp(\Delta att)$/ColE$_1$ $-trp^+$	Y. T. Chi
Phages		
C600/*tet*$_1$	Tn*10* insertion into λ *bb*	C. H. Chung
P$_1$*Kc*	Generalized transduction phage	M. H. Lim

reagent grade. Organic chemicals were of the highest grade commercially available. β -Thienylalanine, 5-methyltryptophan, and p-fluorophenylalanine were obtained from Sigma Chemical Company. [^{3}H]- Tryptophan was purchased from New England Nuclear Co.

Construction of *aroP*$^-$ mutant :Construction of *aroP*$^-$ mutant is discribed in Fig. 1. Tn*10* insertion in the *E. coli* wild type ad P$_1$*Kc* mediated transduction into *the E. coli* HB101 were followed according to the methods in the Manual of Methods for General Bacteriology (1981) and Advanced Bacterial Genetics (1980), respectively.

[^{3}H]-tryptophan uptake by glucose-starved cells: The procedure was followed according to the method of Brown (1970). Vogel Bonner (VB) minimal medium was used in this experiment. Cell suspensions (100ml; 2x10^8 cells/ml) were starved of glucose for 60 min at 37 °C and assayed at 37 °C. Samples (1ml) were removed the medium and filtered through Milipore membrane filters (pore size 0.45 μm, dia. 25mm). These were washed with 10 volumes of medium VB minus glucose prewarmed to 37 °C. Filters were dried, placed in vials containing 10ml of scintillation fluid, and counted in a Beckman LS-250 spectrometer. The scintillation fluid contained, in 1 liter of toluene, 4g of 2,5-diphenyloxazole and 0.1g of 1.4-bis-2-(5-phenyloxazoly)-benzene.

Determination of tryptophan: Tryptophan was determined by the colorimetric method of Udenfriend and Peterson (1957).

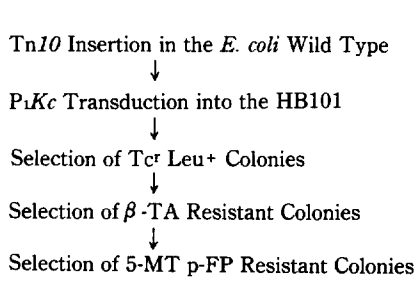

Fig. 1. Diagram for *aroP*$^-$ Mutant Construction.

RESULTS AND DISCUSSION

Isolation of mutant defective in general aromatic transport system: The procedure for obtaining an *aroP* mutation caused by insertion of Tn*10* into the *aroP* region, was based on the known locatin of *aroP* close to the *leu* gene of *E. coli*, and *aroP*$^-$ mutants are resistant to β-thienylalanine. screening Tn*10* insertion strains for β-thienylalanine

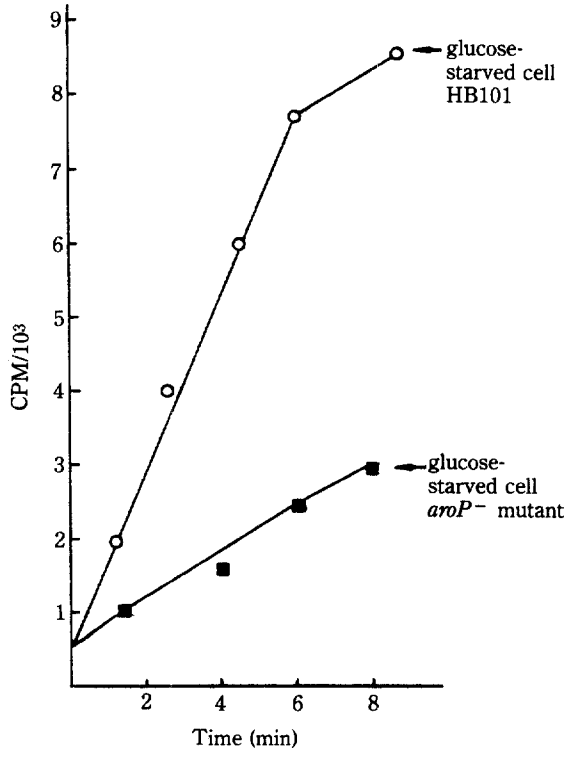

Fig. 2. Patterns of [^{3}H]-tryptophan(10^{-4}M, 1 mCi/mmole) uptake per 10^9 cells at 37 °C

resistncce. A P1 *Kc* generalized transducing phage lysate using a pool of wild type cells with Tn*10* inserted in random locations (W3110::Tn*10*) for growth of bacteriophage, was prepared according to the procedure described in the Material and Method. The resulting P1*Kc* lysate which thus contained a mixture of transducing particles with Tn*10* in different locations, was used to transduce into the *leu*$^-$ recipient strain HB101, selecting Leu$^+$ type on minimal media containing 10 μg/ml tetracycline. These transductants were then screened for β-thienylalanine (2×10^{-4}M) resistance. Of 150 Leu$^+$ and Tet^r transductants tested, 45 colonies showed the β-TA resistance. Thus the phenotype of Th*10*-inserted *aroP* mutation was cotransduced at a frequency of 33% with *leu*$^+$ marker. Of 45 β-TA^r Tet^r colonies, only one colony was resistant to 5-methyltryptophan (2×10^{-4}M) and p-flurorophenylalanine (2×10^{-4}M).

Uptake of [^{3}H]-tryptophan by *aroP*$^-$ mutant: The kinetics of transport of [^{3}H]-tryptophan by glucose-starved *aroP*$^-$ cells is shown in Fig. 2. As shown in Fig. 2, uptake of [^{3}H]-tryptophan by *aroP*$^-$ strain was reduced considerably compared with the rarental otrain.

Therefore, it is likly that general aromatic tranport system of *aroP*$^-$ mutant strain was blocked to that the mutant can not uptake the [^{3}H]-tryptophan from the assay medium by this transport system.

Inhibition of [^{3}H]-tryptophan uptake by azide: Suspensions of glucose-starved *aroP*$^-$ cells and *aroP*$^+$ HB101 cells almost failed to uptake the [^{3}H] tryptophan at 37°C in the presence of sodium azide (NaN_3) (3×10^{-2} M). The results indicate that the amino acid trnsport sistem is an active process coupled to clergy production. As shown in Fig. 3, the rate of [^{3}H]ltryptophan uptake by *aroP*$^-$ mutant strain treated with NaN_3, however, was much less reduced than that by *aroP*$^+$ HB101. These indicate, therefore, that since amino acid transort system is an active process coupled to energy production, in *aroP*$^-$ mutant strain [^{3}H]-tryptophan uptake by active transport is already reduced sufstantially.

Effect of *aroP*$^-$ mutation on tryptophan excretion from *E. coli*: In order to examine the effect of

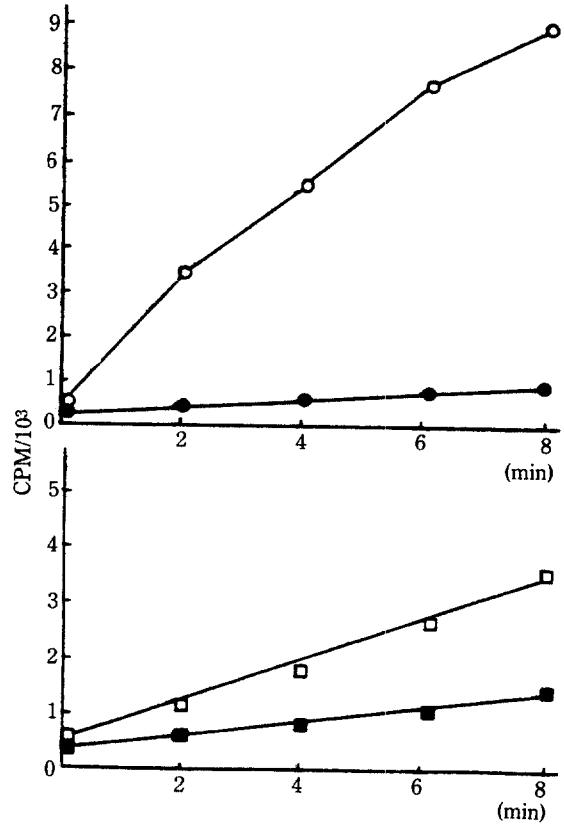

Fig. 3. Patterns of [^{3}H]-tryptophan uptake at 37°C by wild type and *aroP*$^-$ mutant treated with sodium azide
O; Glucose-starved cell HB101
●; Glucose-starved cell HB101 + NaN_3(3×10^{-2}M)
□; Glucose-starved cell *aroP*$^-$ mutant
■; Glucose-starved cell *aroP*$^-$ + NaN_3(3×10^{-2}M)

Table 2. Effect of *aroP*$^-$ mutation on tryptophan excretion from *E. coli trpR*$^{-ts}$ *trpLD*102 (*Δ att*)/pVH5

Strain	Tryptophan concentration in media (mg/l)	
	30℃	42℃
Wild type	2	3
KUB39 (*trpR*$^{-ts}$*aroP*$^+$/pVH5)	6	10
KUB40 (*troR*$^{-ts}$*aroP*$^-$/pVH5)	20	50
KUB41 (*trpR*$^{-ts}$*aroP*$^-$/pVH5)	15	40
KUB42 (*trpR*$^{-ts}$*aroP*$^-$/pVH5)	25	60

All strains were grown in Vogel-Bonner minimal medium for 40hr

aroP$^-$ mutation on tryptophan excretion and the *aroP*$^-$ mutation induced by Tn*10* was transduced into *E. coli trpR*$^{-ts}$/ColE$_1$-*trp*$^+$ cells by bacteriophage P1*Kc and 3 aroP*$^-$ transductants resistant to β-TA, p-FP, and 5MT were isolated and assayed for tryptophan yield. All these transductants showed a higher yield of tryptophan than that of wild type both at 30°C and at 42°C, as shown in Table 2. These results indicate, therefore, that *aroP*$^-$ mutation plays an important role in the transport of tryptophan accumulated within the cell to the medium. Table 2 also shows clearly the effect of *trpR*$^{-ts}$ mutation on tryptophan yield.

적 요

증폭된 재조합 *trp* operon의 발현을 위한 숙주박테리아 개발의 일환으로 숙주 *E. coli*에 *aroP*$^-$ 변이를 도입하였다. *aroP*$^-$ 변이의 유도에는 transposon Tn*10*을 사용하였으며 P1*Kc* 파아지를 이용하여 숙주박테리아에 형질도입하였다. General aromatic amino acid transport system이 결여된 *aroP*$^-$ 변이주는 β-thienylalanine (2×10^{-4}M), p-fluor-phenylalanine (2×10^{-4}M) 그리고 5-methyltryptophan에 저항성을 가졌다. *aroP*$^-$ 변이주는 *aroP*$^+$ 야생주에 비해서 [^{3}H]-tryptophan uptake가 상당히 감소하였다. 또한 NaN_3 (3×10^{-2}M)를 처리하였을 때의 [^{3}H]-tryptophan uptake 비율은 *aroP*$^-$ 변이주가 *aroP*$^+$ 야생주보다 덜 감소하였다. *E. coli trpR*$^{-ts}$/ColE$_1$-*trp*$^-$ 균주에 *aroP*$^-$ 형질을 도입하였을 때 트립토판 방출이 *aroP*$^+$ 야생주에 비해서 4배나 증가하였다.

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