Biochem. J. (1982) **204**, 617–619
Printed in Great Britain

Location of the gene for the low-affinity tryptophan-specific permease of *Escherichia coli*

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(*Received 12 February 1982/Accepted 5 March 1982*)

L-Tryptophan uptake was assayed under conditions in which the *aroT* gene had been inactivated by deletion and the product of the *aroP* permease was competitively inhibited. A mutant carrying a deletion from *bgl* through *tnaA* showed negligible L-tryptophan uptake, in contrast to a strain possessing an intact *tna* region or to strains carrying point mutations in *tna*. The ability to take up L-tryptophan was not restored by lysogenizing the *tna*-deleted strain with λtna^+.

There are at least three transport systems for L-tryptophan in *Escherichia coli*. The first, specified by the gene *aroP*, which lies at 2.5 min (Brown, 1970) on the *E. coli* map (Bachmann & Low, 1980), is a general permease, transporting all three aromatic amino acids. The second, the product of the *aroT* gene (which maps at 27.5 min near the *trp* operon; Kuhn & Somerville, 1971) was originally thought to be specific for tryptophan but (like the *aroP* product) is now believed to be active with all the aromatic amino acids. The third is a low-affinity permease specific for L-tryptophan (Burrous & De Moss, 1963); the location of the gene for this permease is not known. Since this third, low-affinity permease is induced by L-tryptophan and subject to catabolite repression (Burrous & De Moss, 1963), and since this pattern of regulation is similar to that of tryptophanase (an enzyme which degrades L-tryptophan to pyruvate, indole and ammonia), we were interested to see whether the genes for these two proteins were located in the same operon.

As we had reason to believe that transcription of *tnaA*, the structural gene for tryptophanase, was towards the nearby *bgl* operon, and that *tnaA* was immediately adjacent to its promoter (Taylor, 1977), we have examined L-tryptophan transport in a strain carrying a deletion from *bgl* to *tnaA*, to see whether this deletion abolishes the activity of the low-affinity permease.

In order to study the low-affinity permease without interference from the other L-tryptophan permease activities we used strains all of which carry a *tonB–trp* deletion (and which therefore lack *aroT* activity; see Bachmann & Low, 1980), and we competitively inhibited the *aroP* permease by performing the assays in the presence of L-phenylalanine (Whipp & Pittard, 1977).

Experimental

Bacterial strains

The strains used, together with the source or method of construction of each, are listed in Table 1.

Genetic methods

Transductions with phage P1 *clear* were performed as described by Miller (1972). Selection for Tna^+ has been described previously (Yudkin, 1976); $tnaA^+$ *trpAC9* strains can grow with indole as sole source of L-tryptophan provided that the carbon source is only weakly catabolite repressing and that 5-methyltryptophan is present to induce tryptophanase expression. The minimal media used (Yudkin, 1976) were based on that of Vogel & Bonner (1956). Lysogeny with Mu*Cts* was as described by Taylor (1963).

Construction of a bgl–tna deletion

The integrity of the *bgl* region is essential for growth of *E. coli* on salicin. We made deletions extending from *bgl* by isolating a lysogen harbouring Mu*Cts* in the *bgl* operon and then selecting for growth at 42°C. Some of the survivors should carry deletions of Mu*Cts* which could extend into *tnaA*.

Mu*Cts* insertions in the *bgl* operon were isolated by lysogenizing strain MY711 ($bglR^-$) with Mu*Cts*, plating the cells on salicin/eosin/Methylene Blue

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0306-3283/82/050617-03$01.50/1 © 1982 The Biochemical Society

Table 1. *Bacterial strains used in this work*
All strains are *Escherichia coli* of mating type F^-.

Strain	Genotype	Source or method of construction
MY281	*trpAC9–tonB*^{del} *tna2*	This laboratory
MY571	*trpAC9–tonB*^{del}	This laboratory
MY711	*bglR*	This laboratory
MEB6	*bglR tna2 trpAC9–tonB*^{del}	This laboratory
MEB76	*bglR tna106* Val^R *trpAC9–tonB*^{del}	This laboratory
MEB78	*bglR tna108* Val^R *trpAC9–tonB*^{del}	This laboratory
M5	*bglR bgl*::Mu*Cts*	MY711 lysogenized with Mu*Cts*
M11	*bglR tnaA*$^+$ *bgl*::Mu*Cts*	P1 grown on M5 x MEB6
MEB61	*tbna–bgl*^{del} *trpAC9–tonB*^{del}	Survivor of heat shock of M11
MEB64	*tna–bgl*^{del} *trpAC9–tonB*^{del} (λ*tna*$^+$)	MEB61 lysogenized with λ*tna*$^+$ (Borck *et al.*, 1976)

agar at 30°C, and picking non-fermenting colonies, which appeared at a frequency of 0.1%. One such mutant (M5) was confirmed as a *bgl*::Mu*Cts* insertion on the basis of the following observations.

(i) It showed complete lack of reversion to Sal$^+$.

(ii) Phage P1 grown on MY711 was used to transduce M5 to Sal$^+$. All (200/200) of the Sal$^+$ transductants grew at 42°C, which showed that the Sal$^-$ and the temperature-sensitive phenotypes could not be separated.

(iii) Phage P1 grown on M5 at 30°C was used to transduce MEB6 (*tna2 bglR trpAC9*) to grow at 30°C on glycerol/indole/5-methyltryptophan (Yudkin, 1976). Such transductants could be of two classes, *trp*$^+$ or *tna*$^+$. The latter class were identified by their inability to grow with indole as sole source of tryptophan in the absence of 5-methyltryptophan. From the *tna*$^+$ transductants we isolated a Sal$^-$ recombinant (M11). This strain was temperature-sensitive and liberated Mu*Cts* on induction. This observation shows that the site of Mu*Cts* is linked by transduction to *tnaA*.

Strain M11 (*trpAC9 tnaA*$^+$ *bgl*::Mu*Cts*) was grown to stationary phase in L-broth and plated on glucose minimal medium supplemented with L-tryptophan at 44°C. After 36 h survivors were replica plated to glycerol/indole/5-methyltryptophan medium, and a Tna$^-$ survivor was purified (MEB61).

The following properties of MEB61 lead us to believe that it carries a deletion extending from *bgl* to *tnaA*.

(i) It does not revert to Tna$^+$ or Sal$^+$.

(ii) The Sal$^-$ and Tna$^-$ phenotypes could not be separated in P1 transductions (in the wild type, separation between the *tnaA* and *bglR* genes is 30%).

(iii) The *tna* lesion in MEB11 failed to recombine with *tna2* or with any of the nine *tnaA* mutations described by White & Yudkin (1979).

Assay of L-*tryptophan uptake*

The strains were grown overnight in glycerol minimal medium supplemented with L-tryptophan ($100\mu g \cdot ml^{-1}$), diluted into pre-warmed medium to give $A_{600} = 0.2$ and incubated with shaking until the culture density had doubled. The cells from 10 ml of culture were harvested by filtration on a Millipore filter (0.45 μm pore size), washed twice with 10 ml of warm glycerol minimal medium lacking L-tryptophan but containing chloramphenicol ($100\mu g \cdot ml^{-1}$), resuspended in 5 ml of the same medium and kept on ice until they were assayed.

To a 1 ml sample of cell suspension was added 200 μl of L-phenylalanine solution ($5 mg \cdot ml^{-1}$), and the mixture was incubated at 37°C. After 5 min, a 200 μl volume was added of a solution of L-tryptophan {$200\mu g \cdot ml^{-1}$ containing 1×10^6 counts $\cdot min^{-1} \cdot ml^{-1}$ of L-[^{3}H]tryptophan (Amersham)}. The sample was mixed and incubation was continued at 37°C. At 30 s intervals, 100 μl samples were withdrawn; the cells were collected on a Millipore filter held in a 12-position manifold (Millipore), and immediately washed with 2×5 ml of cold minimal medium. Sampling was continued for 5 min. A control sample without cells was included. The filters were dried in a vacuum oven and transferred to scintillation vials. Toluene-based scintillant (5 ml) was added to each vial and the radioactivity was measured in a LKB RackBeta scintillation counter. A_{600} of the cell suspension was determined immediately before the assay and was converted to μg of protein $\cdot ml^{-1}$ by means of a standard curve.

Results

Strains MY571 and MEB61 both carry the *trp* deletion *AC9*; strain MEB61 carries in addition the deletion extending from *tna* to *bgl* that we describe

above. When we measured the low-affinity tryptophan permease in both strains we found that the permease was highly active in strain MY571 but almost undetectable in strain MEB61. It might be argued that the lack of activity in strain MEB61 was due to the absence of tryptophanase from the strain. However, we found that strain MEB64 (which is the λtna^{+} lysogen of strain MEB61 and which synthesizes tryptophanase at 60% of the wild-type rate) had no more permease activity than did MEB61. In both MEB61 and MEB64 the initial rate of uptake was less than 5 nmol of L-tryptophan $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$, whereas in strain MY571 the rate was 80–100 nmol of L-tryptophan $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$.

In subsequent experiments we showed that strain MY281 (which contains the non-reverting mutation *tna2*) behaved in the assay for tryptophan uptake like strains MEB61 and MEB64, whereas strains MEB76 and MEB68 (each of which carries a point mutation in *tna*; White & Yudkin, 1979) behaved like strain MY571. (We found that tryptophan uptake by strains carrying *tna* point mutations could be most clearly observed if the cells were incubated at 37°C in glycerol-minimal medium without tryptophan for at least 15 min before the assay, to deplete the pool of tryptophan.)

Discussion

Our data show that a deletion extending from *bgl* to or beyond *tnaA* causes a loss of L-tryptophan uptake activity under assay conditions that measure specifically the inducible, low-affinity L-tryptophan permease activity. The absence of the permease is not due to the absence of tryptophanase activity; moreover, two point mutants in *tna* retain the permease. There are two possible explanations. The first is that the structural gene for the permease lies in the vicinity of *tnaA*, in a region bounded by *bgl* and the essential gene *dnaA*. The second possibility is that the gene for a positive regulator for the permease has been deleted in MEB61.

If the second alternative were correct, then it would be necessary to propose that the inducible permease and tryptophanase, though showing the same pattern of control, were regulated by different proteins. This is because *tnaA* expression from λtna is regulated normally (i.e. it is inducible and subject to catabolite repression) in the background of strain MEB61 (R. M. Edwards & M. D. Yudkin, unpublished work). We favour the hypothesis that it is the structural gene for the permease that has been deleted.

Our results with strain MY281 prove that *tna2* also abolishes the activity of the specific tryptophan permease. Our mapping data (R. M. Edwards & M. D. Yudkin, unpublished work) show that *tna2* lies within the *tnaA* gene and that it recombines with all the *tnaA* mutations we have tested. We suggest that the permease gene is in the same operon as *tnaA* and that *tna2* has a polar effect on expression of this gene.

We were surprised to find that λtna fails to complement the permease deficiency of strain MEB61, but this failure can now be explained in the light of the DNA sequence for the *tna* region published by Deeley & Yanofsky (1981). These workers have identified an open reading frame downstream of *tnaA* which they suggest is the gene for the inducible low-affinity permease. If the molecular weight of the protein product of this gene were greater than 25000, we can deduce from the data of Deeley & Yanofsky (1981) that the gene would not be carried intact on λtna.

R. M. Edwards was in receipt of a Science Research Council Scholarship for training in research methods.

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