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5-Methyltryptophan-resistant Mutations Linked with the Arginine G Marker in *Escherichia coli*

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G. N. Cohen and F. Jacob (2) described the regulator-constitutive mutants of the tryptophan operon in *Escherichia coli*; these mutants are resistant to 5-methyltryptophan, a structural analogue of tryptophan which inhibits protein synthesis. Other mutants that synthesize the altered anthranilate synthetase, which is insensitive to allosteric inhibition by tryptophan or 5-methyltryptophan, were also found among those resistant to 5-methyltryptophan (8, 10).

We reported that 5-methyltryptophan-resistant mutants independently isolated from strain KY913(HfrH) can be classified into three groups: (i) mutants linked with *thr*, (ii) mutants linked with *cysB* and the tryptophan cluster, and (iii) mutants linked with *argG* (6). The first group of mutants showing the constitutive synthesis of the tryptophan biosynthetic enzymes presumably represent *trpR* mutants. The second group of mutants were found to be operator constitutive (S. Hiraga, J. Mol. Biol., *in press*). In this communication, we report on some properties of the third group of mutants that were found to be cotransduced with *argG* by phage P1 at 60 to 80% (Table 1). The gene or genes for these mutations are termed *mtr* (5-methyl-tryptophan resistance). To test whether the *mtr* mutations have any regulatory effect on the expression of the tryptophan operon, anthranilate synthetase, and tryptophan synthetase (EC 4.2.1.20), activities were measured under conditions of repression and derepression. The synthesis of anthranilate synthetase and tryptophan synthetase was repressible in these mutants (Table 1). In

TABLE 1. *Characteristics of the mtr mutants*

Strain	Cotransduction frequeny with $argG^{+a}$	Anthranilate synthetase[b] (cell extract)		Tryptophan synthase			
				Cell extract		Cell suspension	
		Derepressed	Repressed	Derepressed	Repressed	Derepressed	Repressed
Y-mel(F^+)		100[c]	1.5	100	4.3	100	5
KY913(HfrH)			0.5		4.7	100	5
mtr-4	28/50					93	6
mtr-13	27/50		0.5		11	118	10
mtr-16	40/50				6.2	104	10
mtr-24	30/50		2.5		10	112	9
mtr-25	32/50		2.0		16	104	8
mtr-27	34/50					136	9
mtr-36	37/50					115	6
mtr-4010[d]	75/100					115	8
mtr-4023[d]	81/100					101	3

[a] The cells of strain W4183(*argG*) were infected with phage P1 grown on the *mtr* mutants; the $argG^+$ transductants were isolated and tested for sensitivity to 5-methyltryptophan.

[b] Cells grown in minimal glucose medium (containing 0.2% Casamino Acids) with (repressed condition) or without (derepressed condition) 20 μg of L-tryptophan per ml were harvested, washed, and suspended in tris(hydroxymethyl)aminomethane buffer. Cell suspension or cell extract was used for enzyme assay. Details of the methods are described elsewhere (S. Hiraga, J. Mol. Biol., *in press*.

[c] The values represent per cent of activity in derepressed wild type (Y-mel) or the parental strain (KY913).

[d] The mutations were originally obtained as revertants of the *trpS5* mutant, and were introduced into W4183 by transduction (K. Ito, S. Hiraga, and T. Yura, *in preparation*).

some cases, the activity of tryptophan synthetase under repressed conditions was slightly higher than that of the wild-type strains, although the significance of this is not clear at present.

Some mutants resistant to analogues of an amino acid produce an altered aminoacyl transfer ribonucleic acid (tRNA) synthetase whose affinity to the analogue is reduced (4; I. N. Hirshfield and W. K. Maas, Federation Proc. p. 677, 1967). In particular, in *Salmonella typhimurium*, some of the *his* regulatory mutants resistant to an analogue of histidine produce an altered histidyl tRNA synthetase or tRNA with reduced histidine acceptor activity (9). We reported that the tryptophan-requiring mutation *trpS*, located between *strA* and *malA*, affects the structural gene of tryptophanyl tRNA synthetase (K. Ito, S. Hiraga and T. Yura, *in preparation;* 5–7). Similar mutants were isolated independently in two other laboratories (3; Y. Kano, A. Matsushiro, and Y. Shimura, Mol. Gen. Genetics, *in press*). In contrast to these *trpS* mutants, the *mtr* mutants exhibit normal tryptophanyl tRNA synthetase activity as well as normal tryptophan acceptor activity of tRNA (Table 2). Moreover, tryptophanyl tRNA synthetase activity in the *mtr* mutants was inhibited by 5-methyltryptophan as occurred with the wild-type enzyme.

Mutants having reduced aromatic permease activity are resistant to analogues of tryptophan including 5-methyltryptophan (1; H. S. Moyed and M. Friedman, Bacteriol. Proc. p. 107, 1959). Preliminary experiments revealed no significant differences in the uptake of ^{3}H-L-tryptophan (10^{-6} M) between the *mtr* mutants and the parental strain, with glucose-starved cells as well as growing cells. Permeability to 5-methyltryptophan, however, has not been tested. Thus, the mechanism of 5-methyltryptophan resistance of the *mtr* mutants and its possible relation to the tryptophan operon remain open for future studies.

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TABLE 2. *Tryptophanyl tRNA synthetase and tryptophan acceptor activity of tRNA in the mtr mutants*[a]

Strain	Specific of activity tryptophanyl tRNA synthetase	Tryptophan acceptor activity of tRNA
KY913(HfrH)	100	100
mtr-4	108	118
mtr-4010	117	
mtr-4023	127	

[a] Methods are as described in Ito, Hiraga, and Yura (*in preparation*). The values represent per cent of activiity in hhe parental strain (KY913).

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