Genet. Res., Camb. (1979), **33**, *pp.* 49–55
With 1 text-figure
Printed in Great Britain

Glutamyl-γ-methyl ester acts as a methionine analogue in *Escherichia coli*: analogue resistant mutants map at the *metJ* and *metK* loci

By JAN KRAUS,* DIETER SOLL
Department of Molecular Biophysics and Biochemistry

and K. BROOKS LOW
Department of Therapeutic Radiology, Yale University, New Haven, Connecticut USA 06510

(*Received* 26 *July* 1978)

SUMMARY

Escherichia coli K-12 mutants resistant to glutamyl-γ-methyl ester were isolated. A mutation leading to resistance of up to 1·4 mg/ml of the methionine analogue maps at min 63 and is 13% cotransducible with *serA* indicating an alteration in the *metK* gene. Another mutation leading to resistance to 3 mg/ml of the analogue and cross-resistance to other amino acid analogues maps at min 87. This mutation, which has the phenotype of MetJ$^-$, is shown to be situated between the *glpK* and *metB* genes and thus indicates a different gene order from the published one.

1. INTRODUCTION

Mutants of bacteria resistant to inhibition by analogues of metabolic end products have often been shown to be abnormal in regulation of the biosynthesis of the normal end product (Umbarger, 1971). Among such amino acid analogue resistant mutants, bacteria possessing altered aminoacyl-tRNA synthetases (see e.g. Morgan & Söll, 1978) or tRNA modifying enzymes (Singer *et al.* 1972) have been found. During the search for spontaneous mutants of *E. coli* resistant to several amino acid analogues, we have isolated a number of colonies resistant to glutamyl-γ-methyl ester (= Glu(OME)). Though it was anticipated to be an analogue of glutamate, this compound has been discovered to act as an analogue of methionine. This paper is concerned with the isolation, phenotypic characterization and mapping of these analogue resistant mutants.

2. MATERIALS AND METHODS

(i) *Materials*

L-Glutamic acid γ-methyl ester (Glu(OME)), L-norleucine, L-ethionine, L-methionine-DL-sulphoximine, 3-amino-1,2,4-triazole, L-methionine-DL-sulphoxide,

* Present address: Department of Human Genetics, Yale University.

L-norvaline, DL-5-fluorotryptophan, β-2-thienyl-DL-alanine, 3-nitro-L-tyrosine, DL-*p*-fluorophenylalanine, and 4-methyl-DL-tryptophan were obtained from Sigma Chemical Co., St Louis, Mo. DL-4-azaleucine was supplied by Calbiochem, LaJolla, Ca.

(ii) *Bacterial Strains*

Table 1 gives the characteristics of the strains used in this work.

Table 1. *Bacterial strains*

Strain	Genotype	Source or derivation
D_2	F⁻ *thy-35 strA120*	S. Kaplan (Low *et al.* 1971)
JK13	As D_2 but *metJ108*	Spontaneous Glu(OME)^R from D_2
JK17	As D_2 but *metJ109*	Spontaneous Glu(OME)^R from D_2
JK20	As D_2 but *metK106*	Spontaneous Glu(OME)^R from D_2
KL209	Hfr *sup-53 malB16 thi-1*λ^-	Low, 1973*a*
Ra-2	Hfr *supE42 mal-28* λ^-	Low, 1973*a*
KL14	Hfr *thi-1 relA1* λ^-	Low, 1973*a*
KL16	Hfr *thi-1 relA1* λ^-	Low, 1973*a*
BW113	Hfr *metB1 relA1* λ^-	Low, 1973*a*
AB1157	F⁻ *thi-1 thr-1 leuB6 proA2 argE3 his-4 lacY1 galK2 xyl-5 mtl-1 ara-14 strA31 supE44 tsx-33* λ^-	E. A. Adelberg
X7198	F⁻ *ilvD16 glpK1 metB1 argH1 malA1 lacY1 Z4* or *−20 strA8 9* or *17*	J. Beckwith
KL367	F⁻ *thi⁻ his⁻* (*argF?*) (*argI?*) *serA⁻ strA⁻ lac⁻ mal⁻ xyl⁻ gal*$^{-(s)}$	KL163 × PA260R9 → Arg⁺ [Str^R]
X407	Hfr (Hayes) *proB⁻* λ^- *thi⁻*	R. Curtiss via A. Ahmed, 1973
X407*metJ36*	As X407 but *metJ36*	A. Ahmed, 1973
KL368	F⁻ *thr-1 leuB6 thi-1 proA2 metK86 serA25 glc-1 lacY1 galK2 mtl-1 xyl-5 ara-14 strA25 his⁻* λ^-	KL983 × PL8-31 → MetG⁺ [Str^R]
AB347	Hfr *thi-1 thrA1 leuA1 aroC4 strA723*	Russell & Pittard, 1971
JP1449	As AB347 but *gltX351*	Russell & Pittard, 1971
F122/KL110	F122(*argG⁺thy⁺*)/*argG6 thyA23 metB1 leuB6 his-1 lacY1 gal-6 malA1 xyl-7 mtl-2 strA104 recA1 supE44* λ^-	B. Low, 1972

(iii) *Media and Culturing Conditions*

Luria broth and supplemented minimal medium 56/2 (Low, 1973*a*) were used for matings and routine growth of the strains.

(iv) *Genetic Mapping*

Approximate map positions were determined using Hfr strains with points of origin distributed around the map (Low, 1973*a*, 1973*b*). Transductions were carried out by using P1*vir* (Low *et al.* 1971).

3. RESULTS

(i) *Isolation of Mutants*

Since it was known that Glu(OME) ester supported the ATP-PPi exchange by pure *E. coli* glutamyl-tRNA synthetase (J. Lapointe & D. Soll, unpublished observations), we attempted to isolate mutants resistant to this analogue. Our

hope was to obtain strains with an altered glutamyl-tRNA synthetase which would further the genetic analysis of this enzyme (Morgan & Söll, 1978). For this reason spontaneous mutants of strain D2 were isolated on minimal agar plates containing 300 μg/ml Glu(OME) at 37 °C. This analogue concentration was lethal to strain D2 under these conditions. Analogue resistant revertants were isolated at a frequency of one in 10^8.

(ii) *Mapping with Hfr Strains*

By crossing the mutants with various Hfr's (Low, 1973*a*) and analysing groups of Thy^+ Str^R recombinants for loss of analogue resistance, the approximate genetic locations of the loci conferring resistance to Glu(OME) ester in strains JK13 and JK20 were determined. For use with Hfr KL14, a Mal^- (74 min map position) derivative of JK20 was used, and Mal^+ Str^R recombinants were selected using delayed addition of streptomycin (data not shown). The locus for analogue resistance in strain JK13 was found to be between the points of origin of Hfr strains KL209 and Ra-2 and in strain JK20 between the points of origin of strains KL14 and KL16. These positions correspond approximately to the linkage map segments located between 87 and 90 min and between 61 and 67 min for JK13 and JK20, respectively (Bachmann, Low & Taylor, 1976; Low, 1973*a*). In agreement with the data obtained for JK20, the F′ factor F122 (Low, 1972) was found to bring in the wild type phenotype when crossed with this strain.

Table 2. *Frequencies of cotransduction of* metJ *and* metK *with known loci*

Cross number	Donor*	Recipient*	Selected marker	Number of transductants tested	Number of $Glu(OME)^R$	Frequency of cotransduction	Recombinant classes
1	JK13(*metJ*⁻)	BW113(*metB*⁻)	Met^+	66	59	89 %	
2	JK13(*metJ*⁻)	AB1157(*argE*⁻)	Arg^+	117	37	32 %	
3	JK13(*metJ*⁻)	X7198(*glpK*⁻ *metB*⁻ *argH*⁻)	Met^+	171	169	99 %	*glp*⁻*metJ*⁻*arg*⁻ (47) *glp*⁺*metJ*⁻*arg*⁺ (41) *glp*⁺*metJ*⁻*arg*⁻ (54) *glp*⁻*metJ*⁻*arg*⁺ (27) *glp*⁻*metJ*⁺*arg*⁺ (2)
4	JK20(*metK*⁻)	KL367 (*serA*⁻)	Ser^+	31	4	13 %	

* For full genotypes, see Table 1.

(iii) *Mapping of the Mutations by Transductions*

P1 phage was grown on strains JK13 and JK20 and used to transduce several recipients. The results of these transductions are summarized in Table 2. The proximity of the JK13 locus (leading to Glu(OME) resistance) to the *metJ* gene, together with the correspondence in phenotype to known metJ mutants (see below) led to our tentative assignments of the mutations in JK13 to the *metJ* gene. The cotransduction frequencies between *metB* and *metJ* document a tight linkage of these two loci. The orientation of *metJ* with respect to *glpK* and *arg*

https://doi.org/10.1017/S0016672300018152 Published online by Cambridge University Press

genes was inferred from the 4-point transduction cross (Fig. 1), which indicates the sequence *glpK metJ metB arg*. The location of *metJ* counter-clockwise from *metB* is indicated by the fact that there were no MetJ+ among the 95 MetB+ GlpK+ transductants (Table 2, cross 3) as compared to 2 metJ+ out of 70 MetB+ Arg+ transductants.

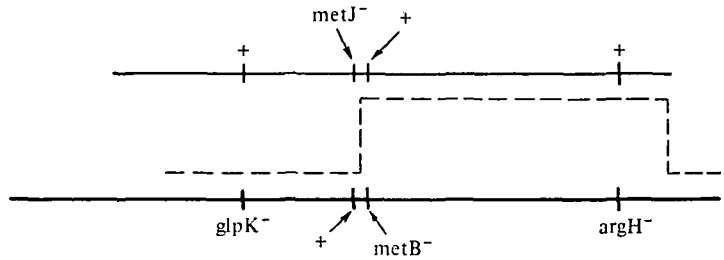

Fig. 1. Illustration of the 4-point transduction cross presented in Table 2 (cross number 3). The donor fragment is represented by the top line and the recipient chromosome by the bottom line. The dashed line illustrates the inferred rare cross-over class of the MetB+ (Glp⁻MetJ+Arg+) transductants.

The mutation resulting in lower resistance to Glu (OME) ester and no resistance to other amino acid analogues is 13% cotransducible with the *serA* locus situated at min 62. Both the frequency of cotransduction with *serA* and the phenotypic similarity to another metK mutant (Table 3), strongly indicate that the mutation has occurred in the *metK* gene (Bachmann *et al.* 1976; Hafner, Tabor & Tabor, 1977). The order of *metK* with respect to *serA* has not been ascertained by transduction. However, a cross with Hfr KL16 (point of origin at min 61) did not result in any MetK+ Thy+ (Str^R) recombinants, and therefore the *metK* locus is probably located clockwise from min 62 (*serA*) (Low, 1972; Bachmann *et al.* 1976).

(iv) *Resistance and Cross-resistance of MetJ and MetK Mutants to Amino Acid Analogues*

Table 3 summarizes the results of multiple tests of strains JK13 and JK20 with 12 different analogues and their comparison to other known MetJ and MetK mutants. Among methionine analogues Glu(OME) gave the most unambiguous and consistent results, and in addition sensitivity to Glu(OME) was lost in the presence of 30 μg/ml methionine (results not shown). There was a clear quantitative difference in inhibitory levels of this analogue between the MetJ and MetK mutants. The MetK mutants grew well up to a concentration of 1440 μg/ml whereas the MetJ mutants routinely grew on concentrations twice as high. All mutants were resistant to two other methionine analogues, norleucine and ethionine, with no selective difference between MetJ and MetK mutants. Whether or not methionine sulphoximine acts as an analogue of methionine is not clear since methionine or casamino acids did not antagonize its inhibitory effect, in contrast to all the other methionine analogues discussed here. The last analogue of methionine tested, methionine sulphoxide, had no inhibitory effect at a concentration of 4 mg/ml. The reverse effect of 3-amino-1,2,4-triazole deserves mention. This compound partially inhibited the mutants but the wild type controls grew in its presence

extremely well. Strain JK13 was cross-resistant to norvaline, 3-nitrotyrosine, fluorophenylalanine and 4-methyltryptophan. Strain X407*metJ36* showed similar but lower levels of cross-resistance. Strain JK20 was not cross-resistant to the analogues of aromatic amino acids when compared to the parent strain D2. Strain KL368 could not be tested on some analogues due to its requirement for five amino acids which antagonized the effect of the analogues.

Table 3. *Amino acid analogue resistance of various strains**

Analogue (conc: μg/ml)	Strain / Relevant genotype	JK13 (*thy⁻metJ⁻*)	JK20 (*thy⁻metK⁻*)	D2 (*thy⁻*)	X407*metJ36* (*pro⁻metJ⁻*)	X407 (*pro⁻*)	KL368 (*thr⁻leu⁻pro⁻ his⁻ser⁻metK⁻*)
Glutamyl γ-methyl ester (1440–3000)		+	+†	−	+	−	+
Norleucine (2500)		+	+	−	+	−	+
Ethionine (4000)		+	+	−	+	−	+
Methionine sulphoximine (600)		+	+	−	±	−	±
Methionine sulphoxide (4000)		+	+	+	+	+	+
Aminotriazole (800)		±	±	+	±	+	
Norvaline (700)		+	−	−	−	−	
Thienylalanine (150)		−	−	−			
3-Nitrotyrosine (700)		+	±	±	+	+	
4-Azaleucine (1000)		−	−	−			
p-Fluoro-phenylalanine (600)		+	±	±	+	±	
4-Methyl-tryptophan (400)		+	±	±	+	±	

* Colonies were screened and assigned to 3 groups: + normal growth, ± partial inhibition, − inhibition.

† MetK mutants were resistant only to the level of 1440 μg/ml.

DISCUSSION

Glutamyl γ-methyl ester is an analogue of methionine as evidenced by the fact that its action can be antagonized by this amino acid. This is further supported by the fact that our analogue resistant strains are altered in the *metJ* and *metK* loci, which specify genes involved in methionine biosynthesis. Evidence is presented that the *metJ* gene is located between *glpK* and *metB* genes. Since the *metF* gene is located between *metB* and *arg* genes (Bachmann *et al.* 1976), it follows that the sequence of the *met* genes in the 87 min cluster is *metJ metB metF* rather than *metB metJ metF* (Su & Greene, 1971) as it is also shown on the current linkage map

(Bachmann *et al.* 1976). Our results indicate that the sequence *metJ metB metF* in *E. coli* is the same as in Salmonella (Ayling & Chater, 1968). This order of the *met* genes was already indicated by Ahmed (1973) for *E. coli metJ* mutants resistant to ethionine (Table 3, *metJ36*). A second locus conferring resistance to Glu(OME) was shown to be a *metK* mutation mapping close to *serA*.

We have some data on yet another possible mutation giving rise to resistance to Glu(OME). Our results indicate that this mutation is tightly linked to the 52 min *gltX*(ts) locus in strain JP1449 (Russell & Pittard, 1971) and is also cotransducible with low frequency (2/400) with the *aroC* locus in strain AB347 (Russell & Pittard, 1971). Linkage of all three markers, namely the ts character, *aroC*, and analogue resistance has so far not been documented. Possibly this mutant may have an altered glutamyl-tRNA synthetase, since *gltX* is believed to code for the structural gene of the catalytic subunit of this enzyme (Lapointe & Delcuve, 1975).

The data presented in Table 3 show that a mutation in the *metJ* gene, coding for the co-repressor of the methionine biosynthetic pathway (Ahmed, 1973), resulted in resistance to several analogues of aromatic amino acids. This could mean that a mutation in the *metJ* gene may also influence the regulation of other operons or result in marked differences in the free amino acid pools (Clandinin & Ahmed, 1973). Alternatively, some general phenomenon, e.g. methylation of nucleic acids might be abnormal in these mutants.

This work was supported by grants from the National Institutes of Health and the National Science Foundation.

REFERENCES

AHMED, A. (1973). Mechanism of repression of methionine biosynthesis in *Escherichia coli*. I. The role of methionine, S-adenosylmethionine, and methionyl-transfer ribonucleic acid in repression. *Molecular and General Genetics* **123**, 299–324.

AYLING, P. D. & CHATER, K. F. (1968). The sequence of four structural and two regulatory methionine genes in the *Salmonella typhimurium* linkage map. *Genetical Research* **12**, 341–354.

BACHMANN, B. J., LOW, K. B. & TAYLOR, A. L. (1976). Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriological Reviews* **40**, 116–167.

CLANDININ, M. T. & AHMED, A. (1973). Mechanism of repression of methionine biosynthesis in *Escherichia coli*. II. The effect of *metJ* mutations on the free amino acid pool. *Molecular and General Genetics* **123**, 325–331.

HAFNER, E. W., TABOR, C. W. & TABOR, H. (1977). Isolation of a *metK* mutant with a temperature-sensitive S-adenosylmethionine synthetase. *Journal of Bacteriology* **132**, 832–840.

LAPOINTE, J. & DELCUVE, G. (1975). Thermosensitive mutants of *Escherichia coli* K-12 altered in the catalytic subunit and in a regulatory factor of the glutamyl-transfer ribonucleic acid synthetase. *Journal of Bacteriology* **121**, 352–358.

LOW, K. B. (1972). *Escherichia coli* K-12 F-prime factors, old and new. *Bacteriological Reviews* **36**, 587–607.

LOW, K. B. (1973*a*). Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. *Journal of Bacteriology* **113**, 798–812.

LOW, K. B. (1973*b*). Restoration by the rac locus of recombinant forming ability in recB$^-$ and recC$^-$ merozygotes of *Escherichia coli* K-12. *Molecular and General Genetics* **122**, 119–130.

Low, K. B., Gates, F., Goldstein, T. & Soll, D. (1971). Isolation and partial characterization of temperature-sensitive *Escherichia coli* mutants with altered leucyl- and seryl-transfer ribonucleic acid synthetases. *Journal of Bacteriology* **108**, 742–750.

Morgan, S. & Soll, D. (1978). Regulation of the biosynthesis of amino acid: tRNA ligases and of tRNA. *Progress in Nucleic Acid Research and Molecular Biology* **21**, 181–207.

Russell, R. R. B. & Pittard, A. J. (1971). Mutants of *Escherichia coli* unable to make protein at 42 °C. *Journal of Bacteriology* **108**, 790–798.

Singer, C. E., Smith, G. R., Cortese, R. & Ames, B. N. (1972). Mutant $tRNA^{His}$ ineffective in repression and lacking two pseudouridine modifications. *Nature New Biology* **238**, 72–74.

Su, C. H. & Greene, R. C. (1971). Regulation of methionine biosynthesis in *Escherichia coli*: mapping of the metJ locus and properties of a $metJ^{+}/metJ^{-}$ diploid. *Proceedings of the National Academy of Sciences, U.S.A.* **68**, 367–371.

Umbarger, H. (1971). Metabolite analogs as genetic and biochemical probes. *Advances in Genetics* **16**, 119–136.