Control of methionine biosynthesis in *Escherichia coli* K12: a closer study with analogue-resistant mutants

M. K. CHATTOPADHYAY, A. K. GHOSH and S. SENGUPTA*

Department of Applied Biochemistry, Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Calcutta 700032, India

Control of methionine biosynthesis in *Escherichia coli* K12 was reinvestigated by using methionine-analogue-resistant mutants. Norleucine (NL) and α-methylmethionine (MM) were found to inhibit methionine biosynthesis directly whereas ethionine (Et) competitively inhibited methionine utilization. Adenosylation of Et to generate *S*-adenosylethionine (AdoEt) by cell-free enzyme from *E. coli* K12 was demonstrated. Tolerance of increasing concentrations of NL by *E. coli* K12 mutants is expressed serially as phenotypes NL^R, $NL^R Et^R$, $NL^R MM^R$ and finally $NL^R Et^R MM^R$. All spontaneous NL^R mutants had a *metK* mutation, whereas NTG-induced mutants had mutations in both the *metK* and *metJ* genes. The kinetics of methionine adenosylation by the *E. coli* K12 cell-free enzyme were found to be similar to those reported for the yeast enzyme, showing the typical lag phase at low methionine concentration and disappearance of this phase when AdoMet was included in the incubation mixture. NL extended the lag phase, and lowered the rate of subsequent methionine adenosylation, but did not affect the shortening of the lag phase of adenosylation by AdoMet.

Introduction

In *Escherichia coli*, the genes of the methionine regulon are distributed throughout the chromosome (Bachmann, 1983). The regulatory functions of *S*-adenosylmethionine (AdoMet) as co-repressor and of the *metJ* gene product as aporepressor have been confirmed by studying *in vitro* expression of some of the *E. coli* methionine biosynthesis genes (Shoeman *et al.*, 1985 *a, b*). However, none of the *metJ* and *metK* (see Fig. 1) regulatory mutants isolated (Su *et al.*, 1970; Kung *et al.*, 1972; Greene *et al.*, 1970, 1973) were fully decontrolled for methionine production. The non-B_{12}-dependent transmethylation step which is one of the two convergent routes for methionine biosynthesis (Fig. 1), synthesizing the methyl group from serine, was also found to be under the same repressor control (Smith, 1971). The regulatory roles of MetJ protein and AdoMet in the expression of the 5,10-methylenetetrahydrofolate reductase gene (Shoeman *et al.*, 1985*a*) and of AdoMet in the synthesis of serine hydroxymethyltransferase have been demonstrated (Greene & Radovich, 1975, Dev & Harvey, 1984). In this paper, we report a reinvestigation of the methionine control system by studying spontaneously occurring methionine-analogue-resistant mutants of *E. coli* K12, with the hope that mutants thus isolated might have a single locus altered among the large numbers of loci involved in the overall control of methionine biosynthesis and might thereby yield a better understanding of the control mechanism.

Methods

Bacterial strains and growth conditions. Escherichia coli K12 strains (see Table 1) and phage P1 were obtained from the *E. coli* Genetic Stock Center, Yale University School of Medicine, New Haven, CT, USA. Minimal medium was that of Davis & Mingioli (1950) with 1% (w/v) glucose. Cells were grown in shake flasks at 37 °C.

Chemicals. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was obtained from Fluka. DL-Ethionine (Et), DL-norleucine (NL), α-methyl-DL-methionine (MM), *S*-adenosyl-L-methionine (AdoMet), *S*-adenosyl-DL-ethionine (AdoEt) and ATP (sodium salt) were purchased from Sigma. SP-Sephadex (C-25) was obtained from Pharmacia. [^{35}S]Methionine was obtained from the Bhabha Atomic Research Centre, Bombay, India.

Determination of minimum inhibitory concentrations (MICs) of methionine analogues and selection of analogue-resistant mutants. MIC values were determined by dilution in minimal medium. To obtain analogue-resistant mutants in liquid medium, cells from the mid-exponential phase were harvested, washed and suitably diluted with saline, and inoculated into analogue-containing minimal medium (AM) at an initial population of approximately 10^5 cells ml^{-1}, growth being permitted up to 72 h. For mutant isolation on solid medium,
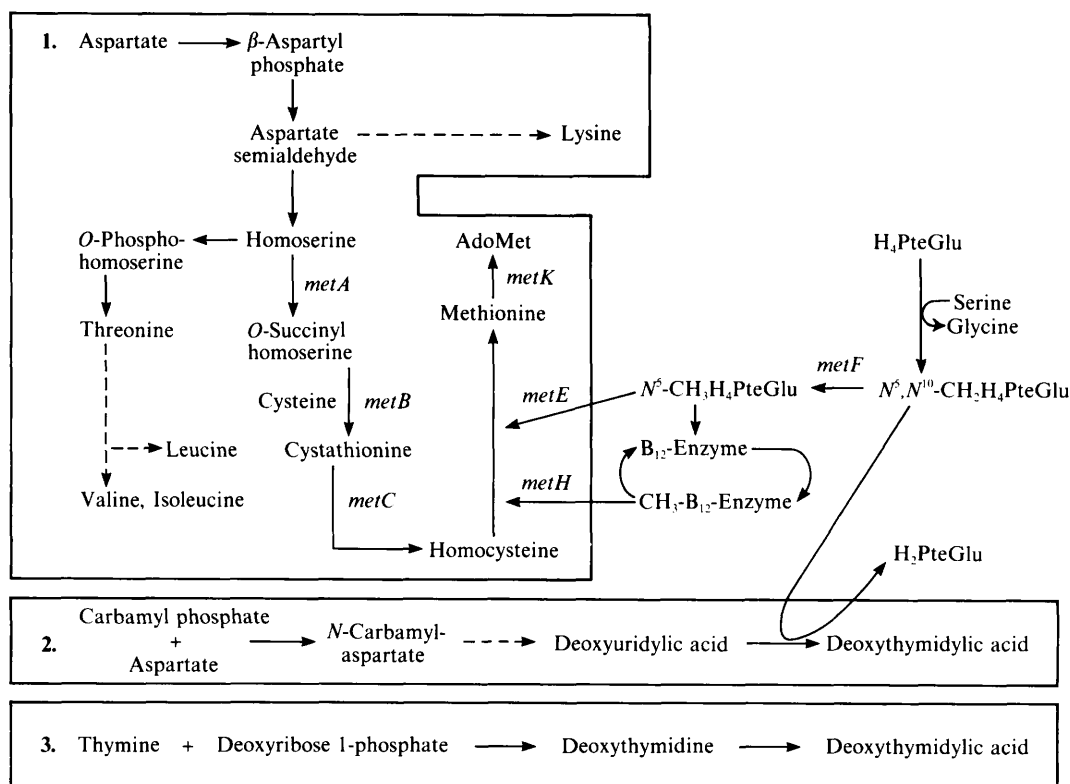
Fig. 1. Outline of the biosynthetic pathways of methionine, methyl group and deoxythymidylic acid in *E. coli*. H$_4$PteGlu, tetrahydropteroyl mono- or triglutamate; AdoMet, *S*-adenosyl-L-methionine. Genes: *metA*, homoserine-*O*-transsuccinylase (EC 2.3.1.46); *metB*, cystathionine-γ-synthase (EC 4.2.99.9); *metC*, β-cystathionase (EC 4.4.1.8); *metE*, N^5-methyltetrahydropteroyl-triglutamate–homocysteine methyltransferase (cobalamine independent) (EC 2.1.1.14); *metF*, N^5,N^{10}-methylenetetrahydrofolate reductase (EC 1.1.99.5); *metH*, N^5-methyltetrahydrofolate–homocysteine methyltransferase (cobalamine dependent) (EC 2.1.1.13); *metK*, methionine adenosyltransferase (EC 2.5.1.6).

Table 1. *Genotypes of recipient strains used for P1 transduction*

Strain	Relevant genotype	Origin
JW383	thr leuB hisG metF zii-510::Tn10 thi Δ(gal-attλ) eda ara lacY xyl mtl rpsL tonA tsx	J. Wechsler, Columbia University, New York, USA
342-167	thr leuB hisG ppc argC thi fhuA lacY supE gal rfbD galP rpsL malA xyl mtl	N. Glansdorf, University of Brussels, Belgium
JM2071	his leu ilvA galP::Tn10 Δlac mglP	H. Kornberg, University of Leicester, UK
JC158	serA thi lacI relA spoT	A. J. Clark, University of California, Berkeley, USA

exponentially growing cells were washed and plated directly on AM agar plates. After 72 h, colonies were transferred to slants of the same medium. NTG mutagenesis was carried out at 30 µg ml^{-1} according to Adelberg *et al.* (1965). NTG-treated cells were grown overnight in AM broth, and the cultures diluted and plated on agar plates of the same medium. Cross-resistance was checked by streaking the overnight growth of mutants on various AM agar plates. Mutant loci of representatives of phenotypic classes of resistant mutants were determined by transduction with phage P1kc according to Miller (1972).

Identification of amino acids. Cells were grown in minimal medium with 1% (w/v) glucose at 37 °C for 48 h and 100 µl volumes of centrifuged culture filtrates were spotted on Whatman no. 1 paper. Ascending paper chromatography was then carried out with n-butanol/acetic acid/water (4:1:1, by vol.). Amino acids were detected with ninhydrin reagent.

ATP:L-methionine-S-adenosyltransferase (methionine adenosyltransferase, EC 2.5.1.6) assay. (a) *Enzyme source.* Cell extracts containing the enzyme were obtained by ammonium sulphate fractionation of cell-free lysates of *E. coli* K12 (Tabor & Tabor, 1971). Cells were grown in minimal medium containing 0·2% (w/v) glucose for 10–12 h. The harvested cells were suspended in glucose-free minimal medium (25 g wet wt per 100 ml) and the suspension was sonicated at an amplitude of 10 µm for 15 min. The lysate was centrifuged at 13000 *g* for 15 min; 5% (w/v) streptomycin sulphate solution was added to the supernatant in equal volume and the precipitate discarded. The supernatant was mixed with half its volume of saturated ammonium sulphate solution, and the mixture left for 1 h with occasional stirring. The supernatant after centrifugation was further mixed with half its volume of saturated ammonium sulphate solution and the mixture left for 45 min and then centrifuged. The final precipitate was collected, dissolved in 0·01 M-potassium phosphate buffer, pH 7·2, and the solution used as the source of enzyme. All the steps were carried out at 4 °C.

(b) *Enzyme assay.* Enzyme activity was assayed in two sets of duplicates. The incubation mixture comprised 0·5 ml of 0·21 M-triethanolamine sulphate buffer, pH 8·2, containing 3·87 µmol methionine, 8·0 µmol ATP, 6·0 µmol NaCN, 30 µmol MgSO$_4$, and cell

extract (40 µg protein). Incubation was carried out for 30 min at 37 °C, the reaction being terminated by the addition of 1·1 ml ice-cold 0·34 M-HCl. The incubation mixtures were kept on ice and applied to an SP-Sephadex (C-25) column (6 mm × 60 mm). Reaction products (adenosylated methionine or ethionine) were eluted from the column by 500 mM- HCl (Glazer & Peale, 1978) and estimated by their absorbance at 260 nm using a Beckmann Spectrophotometer.

Adenosylated amino acids were characterized by paper chromatography and UV spectrometry and compared to authentic samples. Solvent systems (Park, 1958) used in paper chromatography were n-butanol/glacial acetic acid/water (60 : 15 : 25, by vol.) and ethanol/glacial acetic acid/water (65 : 1 : 34, by vol.).

The effects of NL or AdoMet on the kinetics of methionine adenosylation by the enzyme were studied using the same incubation mixture, containing [^{35}S]methionine (0·92 Ci mol^{-1}; 34 GBq mol^{-1}). After 10 min preincubation, reactions were initiated by the addition of methionine; they were terminated at various intervals. Fractions containing AdoMet were eluted from an SP-Sephadex column by 500 mM-HCl and the amount of radioactivity present in each fraction was measured by liquid scintillation counting in a Beckman LS 1800 counter using Bray's solution. Blank tubes were similar except that [^{35}S]methionine was added after the addition of 1·1 ml of ice cold 0·34 M-HCl. Amounts of radioactivity present in the experimental tubes were always corrected by subtracting the amount of radioactivity present in the blanks. Protein was estimated by the Lowry method.

Results and Discussion

Inhibition of the growth of E. coli K12 by methionine analogues

Although mutants of *E. coli* surviving in the presence of Et, MM and NL were characterized long ago as methionine biosynthetic regulatory mutants, the exact mechanism of action of these analogues, other than that of MM, on methionine biosynthetic enzymes (Schlesinger, 1967) was not definitely known. MICs of these analogues for *E. coli* K12 were found to be 3·0 µg ml^{-1}, 4–5 µg ml^{-1} and 2–2·5 mg ml^{-1} for MM, NL and Et respectively. The MICs of NL and MM increased exponentially with increase in inoculum size, while that of Et did not alter significantly under similar conditions (Fig. 2). On the other hand, when L-methionine at concentrations of 7–8 µg ml^{-1} was added to plates containing NL and MM at various concentrations (up to 50 µg ml^{-1}), about 50% colonies were generated compared to analogue-free control plates (1 ± 0·02 × 10^3 colonies per plate). Under similar conditions, graded amounts of L-methionine from 150 µg ml^{-1} to 1 mg ml^{-1} had to be added to plates to restore approximately 50% colony generation in the presence of 2–4 mg Et ml^{-1}. These observations indicated that NL and MM probably inhibited methionine biosynthesis in *E. coli* directly, so that supplementation of the required amount of methionine was sufficient to antagonize activity of NL and MM even in 10–15-fold excess of their growth inhibitory concentrations. However, the higher concentrations of L-methionine required to antagonize the activity of Et
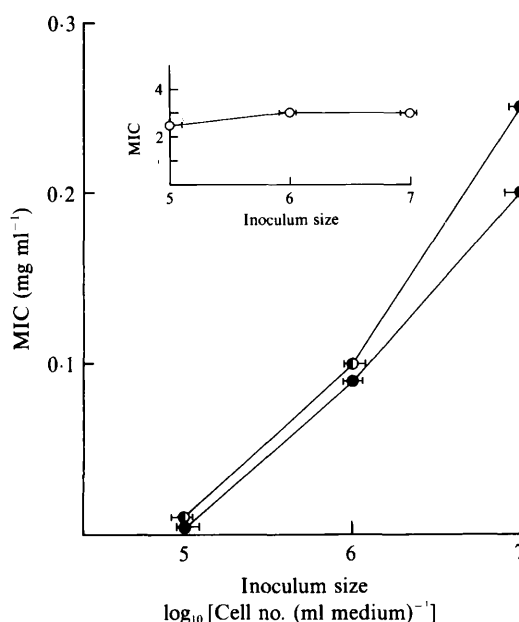

Fig. 2. Variation in MIC values of methionine analogues with changes in inoculum size. *E. coli* cells were grown at 37 °C in minimal medium in shake flasks for both inoculum preparation and growth. A 1 % (v/v) inoculum was added to each analogue-containing medium and growth was measured at 660 nm. ●, MM; ◑, NL; ○, Et. The error bars indicate ± SD of three sets of duplicate experiments. Units for the inset are the same as those for the main figure.

suggested that Et probably did not inhibit methionine biosynthesis directly, but competitively inhibited utilization of methionine. AdoMet may be considered as one of the essential metabolites derived by the cell from methionine. In this context, it may be noted that Et was reported to be freely adenosylated by yeast methionine adenosyltransferase (Park, 1958), but not by the cell-free enzyme from *E. coli* (Peterkofsky, 1965).

AdoEt formation by E. coli K12 enzyme

Reports on the adenosylation of Et by *E. coli* enzyme are contradictory. Martin & Moo-Pen (1963), in their studies on Et resistance of *E. coli*, concluded that the organism could adenosylate Et. However, Peterkofsky (1965) could not detect AdoEt formation by *E. coli* cell-free lysate. In view of the above suggestion that Et may compete with methionine in the synthesis of an essential methionine-derived product, we reinvestigated Et adenosylating activity in an *E. coli* cell extract. Fig. 3 shows the kinetics of adenosylation of Et and/or methionine by the enzyme as a function of amino acid concentration. The results indicated that the enzyme from *E. coli* K12 could freely adenosylate Et, though at a slightly lower rate than methionine. Thus Et might not compete with methionine in the formation of AdoMet in *E. coli*, but the adenosylated product AdoEt might compete with the
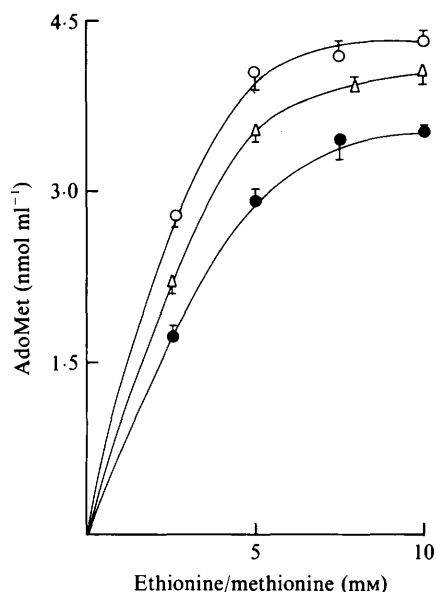
Fig. 3. Adenosylation of Et and methionine by cell-free extract of *E. coli* K12. Reaction mixture contained variable amounts of Et, of L-methionine, or of an equivalent mixture of both, 8·0 μmol ATP, 6·0 μmol NaCN, 30 μmol MgSO$_4$, and enzyme-containing protein (40 μg) in 0·5 ml 0·21 M-triethanolamine sulphate buffer, pH 8·2. The mixtures were incubated for 30 min at 37 °C. Reaction was terminated by the addition of 1·1 ml ice-cold 0·34 M-HCl and adenosylated ethionine and/or methionine were isolated by SP-Sephadex chromatography as described in Methods. Adenosylated amino acids were estimated by determining absorbance at 260 nm ($\varepsilon = 15400$ l mol^{-1} cm^{-1}). O, L-methionine; △, equimolar mixture of L-methionine and Et; ●, Et. The error bars indicate ±SD of three sets of duplicate experiments.

corepresser AdoMet for the binding site of the aporepressor.

Phenotypes of spontaneously occurring methionine-analogue-resistant mutants of E. coli K12

The methionine regulatory *metJ* and *metK* mutants and feedback resistant *metA* mutants of *E. coli* were known long ago. The phenotypes of *metK* mutants were of both methionine overproducer and non-producer types, whereas *metJ* mutants were all overproducers (Smith, 1971). However, the mechanisms for the survival of *metJ* or *metK* mutants in the presence of Et or other analogues were not clear. In the present study we attempted to analyse the phenotypes of all spontaneously occurring methionine-analogue-resistant mutants of *E. coli* K12 as a function of their analogue tolerance limits and spectra.

It was observed (Table 2) that MM and Et specifically selected two groups of mutants with phenotypes similar to those of *metA* and *metJ* mutants. None of the MM^R mutants overproduced methionine or any other amino acid, though they were expected to be feedback-resistant for methionine biosynthesis (Schlesinger, 1967). In the situation when repression control of methionine biosynthesis in *E. coli* K12 is more effective than feedback control, or end-products other than methionine are required for feedback control, overproduction of

Table 2. *Cross-resistance and amino acid overproduction by the analogue-resistant mutants of E. coli K12*

On average, 15–20 colonies of each mutant type were tested for analogue cross-resistance and also for amino acid production in minimal medium. Mutants resistant to Et had two types of amino acid excretion patterns, whereas mutants with lower NL tolerance (25 μg ml^{-1}) excreted Thr and Met only. Frequency was determined for all the survivors irrespective of their subclasses. Lys, lysine; Thr, threonine; Met, methionine.

Analogue (μg per ml of selection medium)	Mean frequency of occurrence*	Cross-resistance type (maximum tolerance dose, μg ml^{-1})	Amino acid excretion patterns
Et (2000) (3000)	$2 \pm 0.5 \times 10^{-5}$	Et (3000)	(i) Lys, Thr, Met (ii) Thr, Met
MM (10), (20), (50)	$3.02 \pm 1.1 \times 10^{-7}$	MM (50)	Nil
NL (10)	$3 \pm 0.2 \times 10^{-7}$	(i) NL (25) (ii) NL (50), Et (3000) NL (50), MM (50) NL (50), MM(50), Et (3000)	(i) Thr, Met (ii) Lys, Thr, Met
NL (25)	$8 \pm 0.8 \times 10^{-7}$	NL (50) NL (50), MM (50) NL (50), MM (50), Et (3000) NL (50), MM (50), Et (4000)	(i) Thr, Met (ii) Lys, Thr, Met
NL (50)	$7.2 \pm 1 \times 10^{-8}$	MM (50), NL (100), Et (3000)	(i) Thr, Met (ii) Lys, Thr, Met
NTG induced (30 μg ml^{-1})		MM (50), NL (100), Et (3000) MM (50), NL (100), Et (4000)	(i) Thr, Met (ii) Lys, Thr, Met

* Means of three determinations, ± SD.

Table 3. *Mapping of mutations in the methionine analogue resistant mutants of E. coli K12*

Donor strains were representative mutants of *E. coli* K12 with different phenotypes within the mutant subgroups as described in Table 2. More than 100 colonies of transformants for each set of experiments were screened for the unselected marker. Three different clones of each phenotypic class were used as donors in each set of experiment.

Donor phenotype	Recipient		Selected marker	Unselected marker	Linkage (%)
1. Et_{3000} (*metF*$^+$ *ppc*$^+$)	JW383	(*metF*, Et^S)	Met$^+$	Et^R	94
	342-167	(*ppc*, Et^S)	Asp$^+$	Et^R	92
2. MM_{50} (*argC*$^+$ *thi*$^+$)	342-167	(*argC*, MM^S)	Arg$^+$	MM^R	34
	JW383	(*thi*, MM^S)	Thi$^+$	MM^R	80
3. NL_{25} (*galP*$^+$ *ser*$^+$)	JM2071	(*galP*, NL^S)	Gal$^+$	NL^R	84
	JC158	(*serA*, NL^S)	Ser$^+$	NL^R	87
4. $NL_{50}MM_{50}$ (*galP*$^+$ *ser*$^+$)	JM2071	(*galP*, NL^S)	Gal$^+$	NL^R MM^R	84
	JC158	(*serA*, NL^S)	Ser$^+$	NL^R MM^R	90
5. NL_{50} Et_{3000} (*galP*$^+$ *ser*$^+$)	JM2071	(*galP*, NL^S)	Gal$^+$	NL^R Et^R	89
	JC158	(*serA*, NL^S)	Ser$^+$	NL^R Et^R	87
6. $NL_{50}MM_{50}Et_{3000}$ (spontaneous) (*galP*$^+$ *serA*$^+$)	JC158	(*serA*, NL^S)	Ser$^+$	NL^R MM^R Et^R	89
7. $MM_{50}NL_{100}Et_{4000}$ (*metF*$^+$ *serA*$^+$)	JW383	(*metF*, Et^S)	Met$^+$	NL^S MM^S Et^R	91
	JC158	(*serA*, NL^S)	Ser$^+$	NL^R MM^R Et^R	84

methionine may not take place in these mutants. Thus, the involvement of AdoMet in feedback inhibition of homoserine transsuccinylase, as reported by Lee *et al.* (1966), was supported by our investigation.

The *metJ* mutants were methionine overproducers but were sensitive to feedback inhibition exerted by the methionine analogue MM. However, these mutants did not accumulate homoserine in the culture filtrate in spite of constitutive expression of the methionine biosynthetic enzymes. We have observed that *E. coli* K12 could adenosylate Et to generate AdoEt which might act as a false corepressor. Any mutation which altered the structure of MetJ protein, affecting its binding either to AdoMet or to operator DNA in its active repressor form, would be selected in presence of Et. Thus the *metJ* mutant would have more AdoMet in the cell pool, as reported by Meedel & Pizer (1974). If both AdoMet and methionine are involved in the feedback inhibition, MM could inhibit growth of *metJ* mutants when there was sufficient AdoMet present in the cell pool.

The phenotypes of mutants surviving in the presence of increasing concentrations of NL were found to be distinctly different (Table 2): (i) mutants which could tolerate limited concentrations of NL had mixed phenotypes such as NL^R, NL^{R}Et^R or NL^{R}MM^R, and (ii) mutants with higher NL tolerance had mostly NL^{R}Et^{R}MM^R phenotypes similar to *metK* mutants. However, all these NL-resistant phenotypes escaped detection in Et- or MM-containing plates, possibly due to their lower frequencies of occurrence than *metJ* and *metA* mutants (Table 2).

Mutagenesis of *E. coli* K12 by NTG followed by screening of the survivors in the presence of all the different concentrations of MM, Et and NL, predominantly selected MetK$^-$ phenotype mutants irrespective of the analogue or the concentration used for screening. It appeared that the MetK$^-$ phenotype was the ultimate for mutants surviving in the presence of high concentrations of methionine analogues. It was subsequently observed (see below) that most of the NTG-induced *metK* mutants were genetically different from the spontaneously occurring mutants of similar phenotype (Table 3) in having an additional mutation in the *metJ* gene.

Characterization of genotypes of methionine-analogue-resistant mutants

It was observed that the spontaneous Et^R mutants were of *metJ* genotypes, since their resistance marker was more than 90% co-transducible with the *metF* (89 min) and *ppc* (89 min) genes during P1 transduction (Table 3). MM^R mutants selected in the presence of MM had a *metA* genotype, since their resistance marker was 80% co-transducible with *thi* (90 min), but only 34% with *argC* (89 min). Tolerance of NL in the spontaneous *E. coli* K12 mutants was solely linked to *metK*, since the resistance markers of the phenotypes NL^R, NL^{R}MM^R, NL^{R}Et^R or NL^{R}Et^{R}MM^R were co-transduced simultaneously with *serA* (63 min) and *galP* (64 min). Thus all the spontaneous NL^R mutants with different phenotypes belong to the same *metK* genotype. However, NL^{R}MM^{R}Et^R mutants obtained by NTG mutagenesis were interesting. One group was characterized as *metK*

whereas another group was found to have mutations at both *metJ* and *metK* loci. Thus it was found that the mutants selected by MM and Et had mutations in the *O*-(succinyl)homoserine synthase (*metA*) and methionine regulatory (*metJ*) genes respectively. All the mutants selected in presence of NL uniformly had mutations in the methionine adenosyltransferase (*metK*) gene, though they had varied analogue resistance.

Activity of NL on in vitro adenosylation of methionine by E. coli K12 enzyme

It was puzzling that NL, which was shown to have no activity on *in vitro* adenosylation of methionine by *E. coli* enzyme (Cox & Smith, 1969), could select mutants with altered enzyme activity for the same reaction (Greene *et al.*, 1973). ATP : L-methionine-*S*-adenosyl transferase of *E. coli* was reported to be different from that of *Saccharomyces cerevisiae*, which was activated by the product AdoMet and inhibited by NL at low methionine concentration (Chou & Talalay, 1972). None of these properties could be detected in the purified enzyme from *E. coli* (Markham *et al.*, 1980).

The present study using spontaneously occurring methionine-analogue-resistant mutants of *E. coli* K12 strongly suggested that NL might have a direct role in the synthesis of AdoMet. All the mutants with different phenotypes selected in presence of NL were found to be *metK* and none of them had any mutation at *metJ* with respect to AdoMet affinity. So the kinetics of AdoMet synthesis were restudied using an enzyme preparation (see Methods). In the synthesis of AdoMet using the same amounts of cell protein (40 μg) and ATP (16 mM), two distinctly different kinetics were observed in presence of two different concentrations of [^{35}S]methionine, 7·77 and 0·77 mM (Fig. 4). Kinetics of [^{35}S]AdoMet synthesis, studied for 60 min with 7·7 mM methionine, were similar to those reported by Markham *et al.* (1980). Presence of NL (20 molar excess over methionine) had no effect on the adenosylation. However, the kinetics changed significantly when the methionine concentration was reduced 10-fold under the same experimental conditions. The kinetics were found to be similar to those observed for the yeast enzyme (Chou & Talalay, 1972). The characteristic lag phase in the synthesis of AdoMet by the enzyme was observed up to 5 min of incubation. The addition of 0·16 mM-AdoMet to the incubation mixture reduced the lag phase to 2 min, and 0·32 mM-AdoMet abolished the lag phase completely. Presence of NL at 20 molar excess over methionine in the incubation mixture affected AdoMet synthesis by extending the lag phase from 5 to 10 min and partially lowering subsequent adenosylation throughout the incubation period, compared to a control containing no NL. Addition of
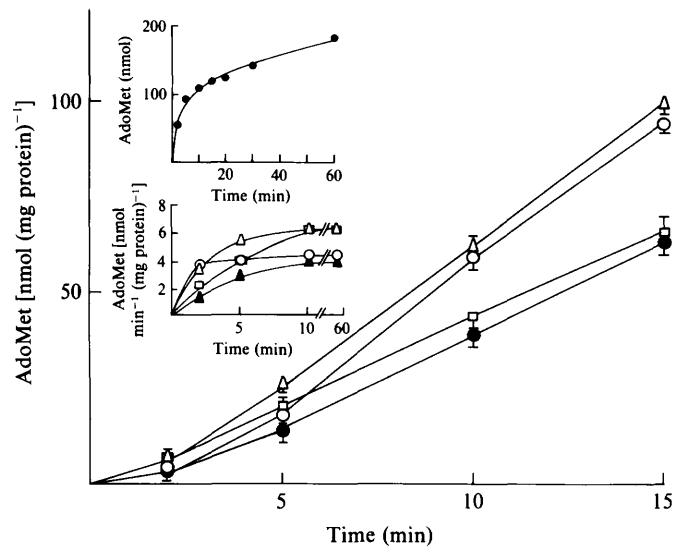

Fig. 4. Kinetics of *S*-adenosylation of L-methionine by the *E. coli* K12 enzyme in the presence of NL. The reaction was conducted with two different concentrations (7·7 mM and 0·77 mM) of [^{35}S]methionine (0·92 Ci mol⁻¹). Other components of the reaction mixture were as described in the legend to Fig. 3. NL was added in 20 molar excess (15·4 mM) over methionine. AdoMet concentration was 0·16 mM. The reaction was terminated at various times and [^{35}S]Adomet generated was recovered as described in Materials and Methods. O, methionine (0·77 mM); △, Adomet (0·16 mM) + methionine 0·77 mM); □, Adomet (0·16 mM) + methionine (0·77 mM) + NL (15·4 mM). The error bars indicate ±SD of three sets of duplicate experiments. Insets: ●, Methionine (7·7 mM); △, AdoMet (0·16 mM) + methionine (0·77 mM); □, methionine (0·77 mM); O, AdoMet (0·16 mM) + methionine (0·77 mM) + NL (15·4 mM); ▲, methionine (0·77 mM) + NL (15·4 mM).

AdoMet to the incubation mixture containing NL reduced the lag period for adenosylation to 5 min but did not improve the adenosylation rate lowered by NL. It appeared that NL did not have marked inhibitory activity on the AdoMet activation of the enzyme, but inhibited methionine adenosylation by the enzyme. The enzyme present in *metK* mutants probably had a modified methionine-binding site resistant to interference by NL, but the enzyme thus modified possibly either could not adenosylate methionine at a rate similar to the native enzyme or could not bind the substrate efficiently. Hobson & Smith (1973) either identified *metK* mutants possessing AdoMet synthetase with high K_m or with undetectable activity. Comparative studies of purified enzymes from the wild-type and *metK* mutants might reveal precisely the modifications that can occur in the enzyme.

Conclusions

The observations in the present study may be summarized as follows:

1. Et is adenosylated in *E. coli* K12 to generate AdoEt,

which might be the false co-repressor for the methionine regulon.

2. Tolerance of increasing concentrations of NL by *E. coli* K12 mutants is expressed serially as phenotypes $NL^R \rightarrow (NL^R Et^R$ or $NL^R MM^R) \rightarrow NL^R$, MM^R, Et^R, the genotype being *metK* in all cases.

3. Participation of both methionine and AdoMet in the feedback inhibition of homoserine succinylation in *E. coli* K12 as indicated by Lee *et al.* (1966) was further supported.

4. $NL^R MM^R Et^R$ mutants of *E. coli* K12 consisted of at least two genotypes, *metK* and *metK metJ*.

5. The AdoMet-synthesizing enzyme of *E. coli* K12 is similar to that of *S. cerevisiae,* and methionine adenosylation by *E. coli* enzyme is partially inhibited by NL when methionine is present at low concentration.

6. The phenotypes of different NL resistant mutants affected in *metK* could be explained on the basis of the present investigation. NL affects AdoMet synthase in *E. coli*. NL^R mutants with low AdoMet synthetase could not adenosylate Et efficiently to generate sufficient AdoEt to either repress methionine biosynthesis ($NL^R Et^R$) or effectively inhibit homoserine succinylase with methionine ($NL^R MM^R$) or both ($NL^R Et^R MM^R$).

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