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# Isolation of a *metK* Mutant with a Temperature-Sensitive *S*-Adenosylmethionine Synthetase

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An *Escherichia coli metK* mutant, designated *metK110*, was isolated among spontaneous ethionine-resistant organisms selected at 42°C. The *S*-adenosylmethionine synthetase activity of this mutant was present at lower levels than in the corresponding wild-type strain and was more labile than the wild-type enzyme when heated or dialyzed. A mixture of mutant and wild-type enzyme preparations had an activity equal to the sum of the component activities. These facts strongly suggest that the mutated gene in this strain is the structural gene for this enzyme. Genetic mapping experiments placed the *metK110* mutation near or at the site of other known *metK* mutants (i.e., 63 min), confirming its designation as a *metK* mutant. A revised gene order has been established for this region, i.e., *metC glc speC metK speB serA*.

*Escherichia coli S*-adenosylmethionine synthetase converts methionine and adenosine 5'-triphosphate to *S*-adenosylmethionine (4, 24). Greene et al. (9, 10) have described one class of ethionine-resistant *E. coli* mutants that have low levels of this enzyme and overproduce methionine (the presumed basis for their ethionine resistance). These workers (9, 11), as well as Maas (15), showed that the gene responsible for this phenotype lies near min 63 on the *E. coli* chromosomal map (3), and they designated the gene *metK* (9, 15) by analogy to similar ethionine-resistant mutants of *Salmonella typhimurium* (13). To explain the mutational phenotype, it has been suggested that the *metK* locus is the structural gene for *S*-adenosylmethionine synthetase and that adenosylmethionine (or a metabolite) is the corepressor involved in controlling the enzymes for methionine and adenosylmethionine biosynthesis (9, 11). Thus, *metK* mutants are derepressed for methionine biosynthesis, according to this hypothesis, because they produce low levels of this corepressor. In the present work, evidence to support the postulation that the *metK* gene is the structural gene for adenosylmethionine synthetase was obtained.

## MATERIALS AND METHODS

**Materials.** ICR191D was a gift from H. J. Creech. Jack bean meal urease was from Sigma Chemical Co. Other reagents were from commercial sources.

**Construction of bacterial strains (Table 1).** Medium preparation, P1*vir* transductions, Hfr- and F'-mediated bacterial matings, mutagenesis, selection of *rif* mutants, and the introduction of *thyA* genes into strains were by the well-known procedures described by Miller (17). P1*vir* transductants were selected on minimal media containing only the nutrients required by the recipient. The minimal medium used was that described by Vogel and Bonner (27). Amino acids, when required, were present at 100 μg/ml; spermidine and putrescine were added when indicated at $5 \times 10^{-5}$ M; thiamine hydrochloride was present in all minimal media at 1 μg/ml. *tolC* mutants were isolated as having resistance to colicin E1 and sensitivity to sodium dodecyl sulfate and as showing no reversion to resistance to sodium dodecyl sulfate; *tolC*$^+$ transductants were selected (after phenotypic expression) on agar plates containing 0.5% sodium dodecyl sulfate (29; J. Foulds, personal communication). The supernatant from an overnight culture (in LB medium, 37°C) of strain EWH224 was sterilized by ultrafiltration (Millipore membrane filters). This sterilized preparation was the colicin E1 used in these experiments.

(Genotypes are referred to throughout by the symbols proposed by Bachmann et al. [3] and Demerec et al. [7].)

**Selection and testing of ethionine-resistant mutants.** DL-Ethionine was incorporated into minimal agar plates at concentrations of 42, 20, and 9 mM for selection and testing of ethionine-resistant mutants of strain EWH80 at 30, 37, and 42°C, respectively, as well as for the testing of transductants for inheritance of the *metK* or *metJ* allele. It has been observed here and elsewhere (1) that ethionine is more toxic at higher temperatures. When colonies were tested for ethionine resistance, tests were most clear when the test plates were inoculated lightly with freshly grown cells. *S*-adenosylmethionine synthetase assays of toluenized transductants were done for several of the crosses presented in Table 4; these assays always

TABLE 1. *E. coli K-12 strains*

| EWH strain no. | Relevant properties | Source |
|---|---|---|
| Parent strains | | |
| 80 | F⁻, wild-type, *relA*, Sup⁺ (unmapped) | A. V. Furano |
| 88 | *thi-1 serA6* | JC158, from A. J. Clark via B. J. Bachmann |
| 154 | *thr-1 leu-6 thi-1 proA2 hisC3 metG87 metK86 serA25 glc-1 str-25* | PLS31, from R. C. Greene via B. J. Bachmann |
| 159 | F⁻ *argG6 metC69 his-1 thyA3 str* | AT2699, from A. L. Taylor via B. J. Bachmann |
| 161 | *metB str glc-1* | $RC_1E_1$, from E. Vanderwinkel and M. De Vlieghere |
| 170 | *metK⁺ glc-15 argG6 thyA3 his-1 str* | *metC⁺ glc-15* P1*vir* transductant of EWH159 |
| 188 | Hfr KL16 *recA1 thi* | KL16-99, from K. B. Low via B. J. Bachmann |
| 195 | *thr leu thi speB speC* | MA255 from W. Maas (6) |
| 224 | Produces colicin E1 | JF390, from J. Foulds |
| 234 | *metK85 serA* | MA233 from S. Cunningham-Rundles and W. Maas (6) (*metK85* isolated by R. C. Greene) |
| Ethionine-resistant derivatives (from this work) | | |
| 47 | *metJ47* | Spontaneous *eth*ʳ (37°C) derivative of EWH80 |
| 110 | *metK110* | Spontaneous *eth*ʳ (42°C) derivative of EWH80 (see text) |
| 174 | *metK110 argG6 thyA3 his-1 str* | *glc⁺ metK110* P1*vir* transductant of EWH170 |
| 204 | *metK110 metJ47 argG6 thyA3 rif str recA1* | *rif metJ47* P1*vir* transductant of EWH174[a]; then *his⁺ recA1* recombinant from cross with Hfr KL16-99 (EWH188) |
| 205 | *metK⁺ metJ47 argG6 thyA3 rif str recA1* | Same derivation as for EWH204, but EWH170 was parent |
| 209 | *speC metK110 speB⁺* | Derivative of EWH195 (see text) |
| 218 | *metK110 serA25* | *thyA⁺ serA25* P1*vir* transductant of EWH174 |
| Other strains | | |
| 177 | *glc-11* | *metC⁺ glc-11* P1*vir* transductant of EWH159 |
| 231 | *tolC glc-11* | *metC⁺ glc-11* P1*vir* transductant of EWH159, followed by colicin E1-resistant mutant selection (see Materials and Methods) |
| 228 | *metC69 glc-11* | *tolC⁺ metC69* P1*vir* transductant of EWH231 |

[a] Cotransduction of the *metJ47* allele was detected by in vitro assay for derepressed adenosylmethionine synthetase activity from cells grown in rich (plus methionine) medium (23).

confirmed the ethionine-resistant test for *metK* mutants.

In the search for *metK* mutants with a temperature-sensitive adenosylmethionine synthetase without the requirement that they be conditionally lethal, ethionine-resistant mutants isolated at 42°C were tested for ethionine sensitivity at 30°C by replica-plating grids (28) of the colonies onto the appropriate medium. Candidates that showed reduced growth in the presence of ethionine at 30°C were examined for the presence of a temperature-sensitive enzyme.

Transductants carrying a *metK* mutation in a *metG87* background were detected as described by Hunter et al. (11). (The *metK* mutation, causing derepressed methionine biosynthesis, satisfies the high-methionine-concentration requirement of the *metG* [methionyl transfer ribonucleic acid synthetase-deficient] strain.)

**S-Adenosylmethionine synthetase assay.** S-Adenosylmethionine synthetase activity was measured by a modification of the methods of Chou and Lombardini (5) and McKenzie and Gholson (16). These methods take advantage of the high affinity of the strongly basic S-adenosylmethionine for negatively charged ion-exchange resins (21). The assay mixtures contained extract, adenosine 5'-triphosphate (10 mM), KCl (200 mM), $MgSO_4$ (100 mM), tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (200 mM; pH 8.1, 20°C), and 0.1 μCi of L-[*methyl*-$^{14}$C]methionine (1.1 mM) in a 50-μl volume. After incubation at 37°C for 15 min, each sample was applied to a separate 3-cm square of Whatman WA-2 cation-exchange resin-loaded paper ($H^+$ form), which was subsequently saturated with water. The papers from several assays were placed between two circles of Whatman no. 1 paper, washed on a Büchner funnel with 6 liters of water to remove the unreacted methionine, dried, and counted in a liquid scintillation counter. The radioactivity on the paper, representing S-adenosylmethionine, was corrected for losses (approximately 30%) due to the washing procedure and quenching.

**Assays of bacterial transductants for enzyme activities. (i) Ornithine decarboxylase.** Cultures were grown in Falcon plates containing the appropriate minimal medium (with spermidine). The plates were centrifuged (28), and the cell pellets were suspended in 0.1 M Tris-hydrochloride (pH 8.1) containing 1% toluene (buffer was subjected to sonic oscillation just before use [20]). A 50-μl aliquot of the freshly prepared assay mixture, containing Tris-hydrochloride buffer (pH 8.1; 0.02 M), bovine serum albumin (1 mg/ml), dithioerythritol (5 mM), pyridoxal phosphate (0.05 mM), and L-[*U*-$^{14}$C]ornithine (0.2 mM; 1 μCi/ml) or DL-[1-$^{14}$C]ornithine (0.2 mM; 0.3 μCi/ml), was added to each well. The Falcon plates were covered with $Ba(OH)_2$-impregnated paper and incubated at 37°C for 2 to 3 h. The released $^{14}CO_2$ was trapped as $Ba^{14}CO_3$ and detected by autoradiography (25).

**(ii) Agmatine ureohydrolase.** Agmatine ureohydrolase was assayed in vivo in a coupled reaction system in which L-[*guanido*-$^{14}$C]arginine was converted to $^{14}CO_2$ by the sequential actions of endogenous arginine decarboxylase, endogenous agmatine ureohydrolase, and exogenous urease. A minimal medium was prepared containing the required supplements, spermidine, 0.2% glucose, and crude jack bean meal urease (10 mg/ml). This suspension was centrifuged, and the resulting supernatant was sterilized by ultrafiltration (Millipore). Portions of this clarified medium (100 μl) were placed in the 96 wells of a Falcon tissue culture plate with the use of a semiautomatic dispensing apparatus (Cooke catalog no. 2-325-1). After the wells were inoculated, 1 drop (ca. 25 μl) of 0.3 mM L-[*guanido*-$^{14}$C]arginine (1.0 μCi/ml) was added to each well. Paper impregnated with $Ba(OH)_2$ was placed over the Falcon plate. [$^{14}$C]urea, the product of the endogenous bacterial reactions, was excreted into the medium, where it was converted to $^{14}CO_2$ by the action of urease (18, 19). The $^{14}CO_2$ was converted to $Ba^{14}CO_3$ on the overlying $Ba(OH)_2$ paper. After overnight incubation of the plates at 37°C, the paper was dried and subjected to autoradiography (25).

**Partial purification of S-adenosylmethionine synthetase.** Cells were grown in minimal medium at 37°C to the late logarithmic stage and harvested by centrifugation. A suspension of 23 g of the packed cells in 92 ml of cold (4°C) Tris-hydrochloride (0.1 M; pH 8.1) containing phenylmethylsulfonylfluoride (50 mg/liter), 20% glycerol, KCl (50 mM), $MgSO_4$ (10 mM), and adenosine 5'-triphosphate (0.5 mM) was ruptured by passage through a French press (12,000 $lb/in^2$). The extract was centrifuged at 20,000 × *g* for 1 h, the precipitate was discarded, and to the supernatant solution was added a volume of streptomycin sulfate solution (20%, wt/vol) equal to one-fourth of the supernatant volume. The supernatant solution from the streptomycin precipitation step was fractionated by ammonium sulfate (solid) precipitation. The 37 to 55% fraction (8) was taken up in 15 ml of buffer containing Tris-hydrochloride (pH 7.3; 0.05 M), KCl (0.05 M), glycerol (10%), ethylenediaminetetraacetate (0.001 M), and mercaptoethanol (0.1%) and was dialyzed against 1 liter of buffer of the same composition. After 3 h, the dialysis was repeated with fresh buffer. This preparation was applied to a column (1 by 27 cm) of the cellulose anion exchanger Whatman DE-52 and eluted with a 200-ml linear gradient. The starting buffer (100 ml) contained Tris-hydrochloride (pH 7.3; 0.2 M), and the final buffer (100 ml) contained KCl (0.3 M) plus Tris-hydrochloride (pH 7.3; 0.01 M). Both buffers contained glycerol (10%), ethylenediaminetetraacetate (0.001 M), and mercaptoethanol (0.1%). Fractions of 1.5 ml were collected; the peak of activity appeared in fraction 74 for both mutant and wild-type preparations and was eluted after the bulk of the protein. All steps were carried out at 4°C, and the final fractions were stored at −70°C.

## RESULTS

**Unsuccessful search for a conditionally lethal *metK* mutant.** If the *metK* gene codes for S-adenosylmethionine synthetase and is essential for growth, then it should be possible to isolate a conditionally lethal *metK* mutant. Accordingly, we looked for, but did not find, *metK* mutants that were temperature sensitive for growth by selecting ethionine-resistant mutants at 30°C and screening them for inability to grow at 44°C on minimal medium (no ethionine). In this attempt, several hundred ethionine-resistant colonies were purified by single-colony isolation at 30°C before testing for growth at 44°C, and the rest were tested directly by standard replica-plating techniques using velveteen cloth. Of 3,500 ethionine-resistant colonies screened, 4 did not grow at 42°C, but none of these 4 was a *metK* mutant. Although these four mutants were not *metK* mutants, their detection indicates that the screening method was effective. (The temperature-sensitive mutation in these four mutants is apparently in the *metA* locus, as indicated by a 60 to 70% P1*vir* cotransduction of the mutation with the *rpoB [rif]* gene. Furthermore, it was found that these mu-

tants grow when methionine is added to their medium at 42°C. At 30°C, they were found to excrete [overproduce] methionine, which explains their resistance to ethionine at this temperature.)

Assay (data not shown) of many ethionine-resistant mutants isolated at 30°C in the above-mentioned mutant hunt showed that about 50% were apparently *metK* mutants; i.e., they contained low levels of *S*-adenosylmethionine synthetase. Hence, approximately 1,700 *metK* mutants were tested, but none was temperature sensitive for growth.

**metK mutant containing a temperature-sensitive *S*-adenosylmethionine synthetase.** Since the above-mentioned search did not result in any conditionally lethal *metK* mutants, we looked instead for mutants containing a temperature-sensitive adenosylmethionine synthetase without the requirement that they be conditionally lethal. In this approach, mutants were selected that were resistant to 9 mM ethionine at 42°C but were sensitive to 42 mM ethionine at 30°C (see Materials and Methods). Strain EWH110, one of several such mutants found, was studied in further detail. Genetic mapping experiments (see below) showed that the lesion it contained (designated *metK110*) was indeed in the *metK* region.

**Comparison of the stability of the *S*-adenosylmethionine synthetases in *metK110* and *metK*$^+$ strains.** In crude extracts, the *S*-adenosylmethionine synthetase from a *metK110 metJ47* mutant strain was much less stable to heat and dialysis treatments than was the corresponding wild-type enzyme (Table 2). Similar results (data not shown) were obtained when the strains compared (EWH80 and EWH110) contained a *metJ*$^+$ allele, except that the specific activities measured for these cells, grown in minimal medium (i.e., methionine-free medium that causes incomplete derepression of enzyme biosynthesis), were about one-half those observed for the derepressed *metJ* strains (Table 2). (The *metJ* mutation, which also confers ethionine resistance, apparently causes complete derepression of both adenosylmethionine synthetase and the methionine biosynthetic enzymes [23].)

Partially purified mutant enzyme was also more sensitive than the corresponding wild-type enzyme when incubated at 42°C (Fig. 1 and Table 3). Furthermore, dialysis followed by heating of the purified mutant enzyme preparation results in nearly total loss of activity, again in striking contrast to the much more stable wild-type enzyme (Table 3). These results strongly suggest that the *S*-adenosylmethionine synthetase in the *metK110* strain is structurally different from the corresponding wild-type enzyme.

**Locating the *metK110* mutation on the *E. coli* chromosomal map.** When P1*vir* phage, which was prepared with strain EWH110 (*metK110*) as its host, was used to transduce strain EWH159 (*metC*) to methionine prototrophy, a 6% cotransduction frequency between *metC* and *metK* was observed. Similar experiments (see below) showed 15 to 22% cotransduc-

TABLE 2. *Stability of S-adenosylmethionine synthetase in crude extracts of metK110 and metK*$^+$ *cells*[a]

| Sample | Formation of adenosylmethionine (nmol/min per mg; 37°C) | | | |
|---|---|---|---|---|
| | No preassay treatment | 42°C incubation[b] | Dialysis[c] | Dialysis,[c] then 42°C incubation[d] |
| *metK110* | 1.9 | 0.07 | 0.3 | 0.06 |
| *metK*$^+$ | 5.8 | 5.0 | 5.7 | 5.1 |
| *metK110* + *metK*$^+$ | | | | |
| Observed | 3.9 | 2.8 | 3.2 | 2.6 |
| Expected[e] | 3.9 | 2.5 | 3.0 | 2.6 |

[a] Strains EWH204 (*metK110 metJ47*) and EWH205 (*metK*$^+$ *metJ47*) were grown in LB medium supplemented with thymidine (200 μg/ml) to mid-log phase (ca. 5 × 10$^8$ cells per ml) at 29°C. A 200-ml portion of each culture was centrifuged in the cold. The cell pellet was suspended in cold 0.85% NaCl, centrifuged again, suspended in 5 ml of 0.1 M Tris-hydrochloride (pH 7.3) containing glycerol (20%), KCl (200 mM), and ethylenediaminetetraacetate (1 mM), and then passed through a French press at 20,000 lb/in$^2$. After centrifugation of the preparation at 16,000 × *g* for 30 min, the supernatant was removed, and fractions were treated as indicated and then assayed (5 to 10 μl per assay) for enzyme activity. The protein concentration was approximately 10 mg/ml in all samples.

[b] 80 min of preassay incubation. Heat inactivation of the mutant enzyme could be prevented by the addition of adenosine 5′-triphosphate, $Mg^{2+}$, and $K^+$ at concentrations used in the enzyme assay.

[c] Against 1,000 volumes of 0.1 M Tris-hydrochloride (pH 7.3) at 4°C for 2 h.

[d] 20 min of preassay incubation.

[e] Expected values were calculated assuming additive results from first two lines of data. Equal volumes of the extracts were mixed, and the heating and dialysis were carried out on the mixture.

tion of *metK110* with *serA*, thus placing the *metK110* lesion at or very near the *metK* site (min 63) described by other workers (1, 6, 9, 11, 15). However, our data (see below) indicate an order for the genes in this region that is slightly different from that given recently (6); therefore, our mapping experiments will be described in detail.

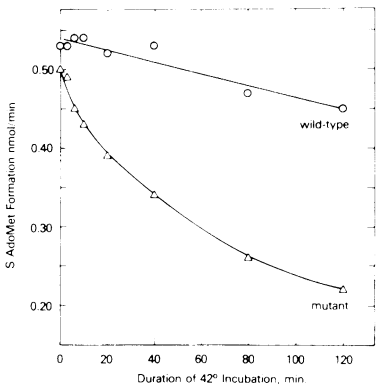


FIG. 1. *Rate of loss of adenosylmethionine (S-AdoMet) synthetase activity of the partially purified mutant enzyme compared to the wild-type enzyme during incubation at 42°C. EWH110 (metK110) and EWH80 (wild-type) were grown at 37°C in minimal medium to the late logarithmic growth stage, and extracts were purified as indicated in the text. The DE-52 fractions with the highest enzyme specific activities (75 and 17 nmol of S-adenosylmethionine formed per min per mg, 37°C, for wild-type and mutant, respectively) were used in this experiment. Each preparation was about 25-fold purified over the crude extract, and the yield for both preparations was approximately 50%. Fractions (0.003 ml) of the wild-type enzyme (1.6 mg of protein per ml) and fractions (0.01 ml) of the mutant enzyme (2.1 mg of protein per ml) were removed from the 42°C incubation and assayed at 42°C. Specific activities for assays at 42°C were approximately 1.5 times those at 37°C.*

In preparation for the mapping experiments given below, strain EWH195 (MA255) (*speC metK*$^+$ *speB*), which grows very slowly on media not containing polyamines (6), was transduced with P1*vir* phage prepared on EWH110 (*speC*$^+$ *metK110 speB*$^+$). Faster-growing polyamine prototrophic transductants (*speC*$^+$ *speB* or *speC speB*$^+$ or *speC*$^+$ *speB*$^+$) were selected and purified (15). During the assay of the purified Spe$^+$ transductants, a *speC metK110 speB*$^+$ recombinant (strain EWH209) was found and was used in the mapping experiments given below.

Quantitative data on the *metK* map position with respect to *speC* and *speB* were obtained in the following experiments. Cross 1 (Table 4) confirmed the *speC speB serA* order given by Cunningham-Rundles and Maas (6). In this three-factor cross and those that follow, it was assumed that recombinants resulting from four crossover events would appear much less frequently than other recombinants that require only two crossover events (footnote *c*, Table 4). This correlation was used to order the genes. The cotransduction frequencies found for *serA* with *speB* (18%) and with *speC* (12%) were also most consistent with a *speC speB serA* gene order.

Evidence for a *speC metK110 serA* gene order was obtained in cross 2 and in cross 3 (ignoring *speB*) (Table 4). Similarly, the frequencies of inheritance in cross 3 of the *speB* and *metK* genes (ignoring *speC*) indicate a gene order of *metK110 speB serA* and, thus, an overall gene order of *speC metK speB serA*. An identical experiment, using as the recipient strain EWH234, which carries the *metK85* allele isolated by Greene and co-workers (11), confirmed

TABLE 3. *Stability of partially purified S-adenosylmethionine synthetase from metK110 and metK*$^+$ *cells*[a]

| Sample[b] | Formation of adenosylmethionine (nmol/min per 5 μl; 42°C) | | | |
|---|---|---|---|---|
| | No preassay treatment | 42°C incubation[c] | Dialysis[d] | Dialysis,[d] then 42°C incubation[e] |
| *metK110* | 0.25 | 0.13 | 0.08 | <0.001 |
| *metK*$^+$ | 0.81 | 0.75 | 0.77 | 0.52 |
| *metK110* + *metK*$^+$ | | | | |
| *Observed* | *0.56* | *0.47* | *0.43* | *0.24* |
| *Expected*[f] | 0.53 | 0.44 | 0.43 | 0.26 |

[a] The partially purified enzymes described in the legend to Fig. 1 were subjected to the treatments shown; then 5-μl samples were assayed. Specific activities can be calculated from the data in Fig. 1.

[b] EWH110 (*metK110 metJ*$^+$) and EWH80 (*metK*$^+$ *metJ*$^+$) were the sources of the enzymes.

[c] 80 min of preassay incubation; see Fig. 1.

[d] Against 1,000 volumes of 0.1 M Tris-hydrochloride (pH 8.1) at 4°C for 3 h.

[e] 40 min of preassay incubation.

[f] Expected values were calculated assuming additive results from the first two lines of data. Equal volumes of the extracts were mixed in this experiment, and then the heating and dialysis treatments were carried out on the mixture.

TABLE 4. *Gene order in the metK region as determined by P1vir transduction*

| | Cross[a] | | | | | EWH strain no. | Selected marker | Transductant genotype class[b] | | | No. in each class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *speC* | *speB* | | |
| | *speC* | *speB* | + | | (donor) | 195 | | + | + | | 68 |
| 1. | | | | | | | *serA*$^+$ | + | − | | 6 |
| | + | + | *serA6* | | (recipient) | 88 | | − | + | | 1[c] |
| | | | | | | | | − | − | | 9 |
| | | | | | | | | *speC* | *metK* | | |
| | *speC* | *metK110* | + | | (donor) | 209 | | + | + | | 138 |
| 2. | | | | | | | *serA*$^+$ | + | − | | 11 |
| | + | + | *serA6* | | (recipient) | 88 | | − | + | | 1[c] |
| | | | | | | | | − | − | | 16 |
| | | | | | | | | *speC* | *metK* | *speB* | |
| | | | | | | | | + | + | + | 0[c] |
| | | | | | | | | + | + | − | 22 |
| | | | | | | | | + | − | + | 129 |
| | *speC* | + | *speB* | + | (donor) | 195 | | + | − | − | 8 |
| 3. | | | | | | | *serA*$^+$ | − | + | + | 0[c] |
| | + | *metK110* | + | *serA25* | (recipient) | 218 | | − | + | − | 7 |
| | | | | | | | | − | − | + | 2[c] |
| | | | | | | | | − | − | − | 0[c] |
| | | | | | | | | *speC* | *metK* | *speB* | |
| | | | | | | | | + | + | + | 3[c] |
| | | | | | | | | + | + | − | 24 |
| | | | | | | | | + | − | + | 137 |
| | *speC* | + | *speB* | + | (donor) | 195 | | + | − | − | 10 |
| 4. | | | | | | | *serA*$^+$ | − | + | + | 3[c] |
| | + | *metK85* | + | *serA25* | (recipient) | | | | | | |
| | | | | | | 234 | | − | + | − | 12 |
| | | | | | | | | − | − | + | 2[c] |
| | | | | | | | | − | − | − | 1[c] |
| | | | | | | | | *speC* | *metK* | | |
| | | | | | | | | + | + | | 58 |
| | + | *speC* | *metK110* | | (donor) | 209 | | + | − | | 2[c] |
| 5. | | | | | | | *glc*$^+$ | − | + | | 14 |
| | *glc-11* | + | + | | (recipient) | 177 | | − | − | | 17 |
| | | | | | | | | *glc* | *speC* | *metK* | |
| | | | | | | | | + | + | + | 55 |
| | | | | | | | | + | + | − | 0[c] |
| | | | | | | | | + | − | + | 12 |
| | + | + | *speC* | *metK110* | (donor) | 209 | | + | − | − | 8 |
| 6. | | | | | | | *metC*$^+$ | − | + | + | 89 |
| | *metC69* | *glc-11* | + | + | (recipient) | 228 | | | | | |
| | | | | | | | | − | + | − | 0[c] |
| | | | | | | | | − | − | + | 2[c] |
| | | | | | | | | − | − | − | 2[c] |

[a] Gene orders shown in this table represent those most consistent with the population frequencies found for the various transductant genotype classes (see footnote *c* and text analysis of cross 1).

[b] +, Inheritance of the wild-type allele; −, inheritance of the mutant allele.

[c] Given the gene orders depicted, low frequencies of transductants with these genotypes are expected because four crossover events are required to generate these transductants. All other transductants need only two crossover events to generate them and, hence, are more frequent.

that *speC metK speB serA* is the gene order (Table 4, cross 4).

To obtain additional evidence for this gene order for the *metK* region, we used the *glc* locus near min 64 on the *E. coli* map (3). Using ICR191D to generate frame-shift mutations (2), we isolated new mutants with the Glc phenotype (unable to use glycolate as a carbon source) (Table 5). Preliminary crosses revealed that the defects in these mutants were located near min 64, as expected (Table 5). Genetic analysis (crosses 5 and 6, Table 4) with one of these mutations, tentatively designated *glc-11*, revealed the gene order *metC glc speC metK*,

confirming our earlier results regarding the order of the *speC* and *metK* genes (Fig. 2).

Finally, additional confirmation of the *glc speC metK* gene order came from our analysis of the *glc-1* allele originally described by Vanderwinkel and De Vlieghere (26) and studied further by Hunter et al. (11). These latter workers found an overall gene order of *metC glc-1 metK serA* and suggested that the *glc-1* allele was a deletion. The evidence we shall now present indicates that *glc-1* is a deletion that includes the *speC* locus but not the *metK* locus. (i) The *glc-1*-containing strains EWH154 (PLS-31) and EWH161 ($RC_1E_1$) have no ornithine decarboxylase activity (the *speC* gene product) and, hence, are presumably *speC*. (ii) *glc*$^+$ revertants of these strains could not be obtained either spontaneously or after mutagenesis with nitrosoguanidine or ICR191D. (iii) Transduction of EWH154 (*glc-1 speC metK86*) to *glc*$^+$ with

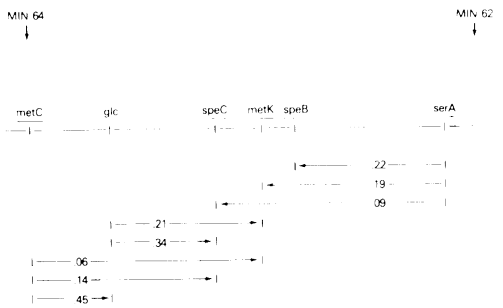


FIG. 2. *metK region of the E. coli chromosomal map. Numbers shown are the cotransduction frequencies of the markers designated by the lines. Arrowheads point away from the selected markers. Data are compiled from Table 5.*

P1*vir* prepared on a wild-type strain restored the *speC*$^+$ gene in all 34 transductants assayed. In our frame-shift *glc-11* mutant, *glc* and *speC* had only a 34% cotransduction frequency (Table 4, cross 5). If the *glc-1* lesion were a point mutation, then only about one-third, and not all, of these 34 transductants should have received the *speC*$^+$ allele. In contrast, only about 20% of the transductants tested received the *metK*$^+$ allele of the donor.

This evidence suggests that the *glc-1* allele is a deletion that includes the *glc* and *speC* regions but not the *metK* locus and implies that the gene order cannot be *glc metK speC*.

A *glc*$^+$ *speC*$^+$ *metK86* transductant from this experiment was used as the recipient in a P1*vir* transduction cross with EWH195 (*speC metK*$^+$ *speB*). Analysis of the recombinants yielded results similar to those found for cross 4, Table 4, confirming the *speC metK speB serA* gene order demonstrated above in the experiments with the *metK110* and *metK85* alleles.

In summary, on the basis of these data we propose that *metC glc speC metK speB serA* is the gene order for this region of the *E. coli* chromosomal map (Fig. 2).

## DISCUSSION

In the present work, we were unable to isolate any conditionally lethal (temperature sensitive for growth) *metK* mutants, although over 1,700 *metK* mutants were examined for this property. Nonetheless, a mutant (EWH110, *metK110*) was obtained that contained a more labile form of *S*-adenosylmethionine synthetase than that found in wild-type cells. This was demonstrated by the much greater inactivation rate of the mutant

TABLE 5. *Frame-shift glc mutants*[a]

| EWH strain no. | *glc* allele | Appearance of *glc*$^+$ revertants | | | Cotransduction frequency (%) of *glc* with: | | |
|---|---|---|---|---|---|---|---|
| | | Spontaneous | Nitrosoguanidine induced | ICR191D induced | *metC*[b] | *serA*[c] | *metK110*[d] |
| 11 | *glc-11* | Yes | No | Yes | 38 | 2 | —[e] |
| 13 | *glc-13* | Yes | No | Yes | 44 | — | — |
| 15 | *glc-15* | No | No | No[f] | — | — | — |
| 170 | *glc-15* | | | | 50 | — | 12 |

[a] ICR191D mutagenesis of EWH80 and the reversion tests of the resulting Glc mutants were performed by the procedures of Miller (17). Glc mutants were detected by standard replica-plating techniques, using minimal agar medium with glycolate (0.25% ammonium glycolate) as the carbon source and supplemented with threonine, leucine, proline, histidine, serine, and thiamine hydrochloride. Among 4,000 colonies screened, 18 Glc mutants were found. The frequency of spontaneous reversion to *glc*$^+$ was approximately $1 \times 10^{-9}$ to $5 \times 10^{-9}$ for *glc-11* and *glc-13*.

[b] Strain EWH159 (*metC*) was the recipient.

[c] Strain EWH88 (*serA*) was the recipient.

[d] Strain EWH170 (*glc-15*) was the recipient.

[e] Transduction was not carried out.

[f] The inability to generate revertants of this mutant suggests that the *glc-15* mutation is a small deletion (2).

enzyme compared with that of the wild-type enzyme during heating or dialysis (Fig. 1 and Tables 2 and 3). Mixing the enzyme preparations from the mutant and wild-type strains yielded the expected additive amount of total activity, even after the above inactivation procedures were performed on the mixture, indicating that the results were not due to the lack of a stabilizing factor or the presence of inhibitors or degradative factors in the mutant (Tables 2 and 3). Thus, these results strongly suggest that *metK* is the structural gene for adenosylmethionine synthetase. A similar conclusion was recently made for the identity of the *metK* gene in *Salmonella typhimurium*, based on the finding of an altered $K_m$ for methionine of the enzyme prepared from a mutant strain (22). The inability to find a conditionally lethal *metK* mutant in *E. coli* may mean that there is more than one gene coding for adenosylmethionine synthetase activity. It is possible that the technique of isolating ethionine-resistant strains at 30°C may select against mutants with a temperature-sensitive adenosylmethionine synthetase, since the enzyme in these mutants is necessarily deficient at 30°C.

In the above search for conditionally lethal (temperature-sensitive), ethionine-resistant mutants, four were found, but they proved to be *metA* mutants instead of the sought-after *metK* mutants. We presume that these *metA* mutants contain heat-labile *O*-succinylhomoserine synthetases (the *metA* gene product) that have lost their ability to be feedback-inhibited by methionine (14). Thus, the mutants overproduce methionine and resist the effects of ethionine at 30°C. As a precedent for this finding, *S. typhimurium* mutants that are α-methylmethionine resistant have been characterized as *metA* mutants that overproduce methionine by the mechanism described above (12, 13). The finding of these mutants, although interesting in its own right, also verifies that the screening methods used to detect temperature-sensitive, ethionine-resistant mutants were effective and supports, although does not prove, the possibility that a second locus codes for *S*-adenosylmethionine synthetase.

A contribution of the present work is the clarification of the gene order in the *metK* region. The *metK110* gene was found to lie between the *speC* and *speB* genes (Fig. 2), near min 63 on the current *E. coli* chromosomal map (3). The *metK85* and *metK86* alleles described by Hunter et al. (11) were also shown in the present studies to reside between *speC* and *speB* (Table 4 and text). This result is in conflict with that of Cunningham-Rundles and Maas (6), who have proposed a *metK speC speB* gene order on the basis of experiments with the *metK85* allele.

Frame-shift mutants unable to grow with glycolate as the carbon source were isolated in this work (Table 5). On the basis of genetic mapping experiments (Tables 4 and 5), we have tentatively assumed that these mutants are affected at the *glc* locus defined by the *glc-1* mutant of Vanderwinkel and De Vlieghere (26). Although the exact defect of glycolate metabolism in these newly isolated frame-shift *glc* mutants and the *glc-1* strains (shown here to be a deletion) is not clear, these mutants proved useful in confirming the gene order found in this work for the *metK* region (Fig. 2).

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