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Influence of Methionine Biosynthesis on Serine Transhydroxymethylase Regulation in *Salmonella typhimurium* LT2[1]

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The enzyme serine transhydroxymethylase (EC 2.1.2.1; L-serine:tetrahydrofolate-5,10-hydroxymethyltransferase) is responsible both for the synthesis of glycine from serine and production of the 5,10-methylenetetrahydrofolate necessary as a methyl donor for methionine synthesis. Two mutants selected for an alteration in serine transhydroxymethylase regulation also have phenotypes characteristic of *metK* (methionine regulatory) mutants, including ethionine, norleucine, and α-methylmethionine resistance and reduced levels of *S*-adenosylmethionine synthetase (EC 2.5.1.6; adenosine 5'-triphosphate:L-methionine *S*-adenosyltransferase) activity. Because this suggested the existence of a common regulatory component, the regulation of serine transhydroxymethylase was examined in other methionine regulatory mutants (*metK* and *metJ* mutants). Normally, serine transhydroxymethylase levels are repressed three- to sixfold in cells grown in the presence of serine, glycine, methionine, adenine, guanine, and thymine. This does not occur in *metK* and *metJ* mutants; thus, these mutations do affect the regulation of both serine transhydroxymethylase and the methionine biosynthetic enzymes. Lesions in the *metK* gene have been reported to reduce *S*-adenosylmethionine synthetase levels. To determine whether the *metK* gene actually encodes for *S*-adenosylmethionine synthetase, a mutant was characterized in which this enzyme has a 26-fold increased apparent K_m for methionine. This mutation causes a phenotype associated with *metK* mutants and is cotransducible with the *serA* locus at the same frequency as *metK* lesions. Thus, the affect of *metK* mutations on the regulation of glycine and methionine synthesis in *Salmonella typhimurium* appears to be due to either an altered *S*-adenosylmethionine synthetase or altered *S*-adenosylmethionine pools.

The pathways for glycine and methionine synthesis are connected, with the methyl group for methionine synthesis coming from serine via the serine transhydroxymethylase reaction (Fig. 1). Thus, serine transhydroxymethylase is essential for both glycine and methionine synthesis. Glycine, 5,10-methylenetetrahydrofolate, and methionine, however, are not the final physiological products, but rather serve as precursors for other compounds, including thymine, purines, *S*-adenosylmethionine, biotin, polyamines, and numerous methylated products (2, 5, 18–21, 28, 29). It is apparent that the serine transhydroxymethylase reaction occurs at a branch point in cell metabolism and that its regulation is important and may be complex.

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Previous work has shown that the addition of purines, pyrimidines, serine, glycine, and methionine to the growth medium results in a reduction of serine transhydroxymethylase activity in both *Salmonella typhimurium* and *Escherichia coli*, suggesting that several compounds are involved in the control of this enzyme (6, 17, 26, 30). Furthermore, in *S. typhimurium*, enhanced repression by the combination of compounds suggests a form of cumulative repression of this enzyme (26). However, whether all of the compounds or only a few are directly involved in the regulatory mechanism has not been determined. Growth of serine, glycine, methionine, and thymine auxotrophs of *S. typhimurium* with limiting concentrations of the respective required compounds did not result in derepressed enzyme levels, which suggests that these compounds do not directly interact in the regulatory mechanism (26). De-

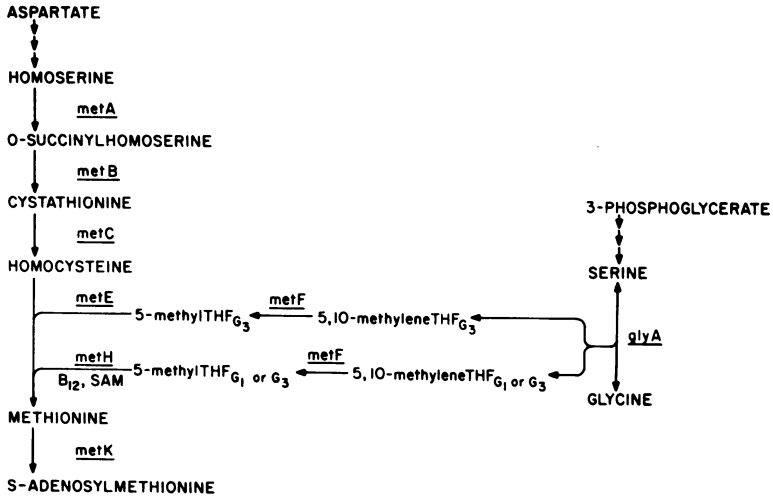

FIG. 1. *Pathway for serine, glycine, and methionine biosynthesis. Abbreviations: 5-methylTHF, 5-methyltetrahydrofolate; 5,10-methyleneTHF, 5,10-methylenetetrahydrofolate; G, or G_3, mono- or triglutamate; SAM, S-adenosylmethionine.*

repression of serine transhydroxymethylase did occur in *S. typhimurium*, however, when a purine auxotroph was starved for purines or when wild-type cells were grown with trimethoprim (26). These results implicate purines and/or folates in the regulation of this enzyme.

Other work suggests that methionine may be involved in the regulation of serine transhydroxymethylase levels in *E. coli* (6, 15). Growth of *metF* and *metE* mutants with limiting concentrations of methionine produced derepressed enzyme levels for serine transhydroxymethylase. Although these results suggest that for *E. coli* methionine is important for serine transhydroxymethylase regulation, they provide no information on a mechanism for a methionine involvement.

As can be seen from the above discussion, a variety of physiological studies have suggested an involvement of purines, folates, and/or methionine in the regulation of serine transhydroxymethylase synthesis but do not identify the component(s) important for the regulatory signal. Because mutant analysis has been a profitable approach for examining the regulation of other pathways, we devised a selection procedure to isolate mutants having altered serine transhydroxymethylase levels. Two of these mutants simultaneously became resistant to methionine analogues, and their characterization has focused on the connection between serine transhydroxymethylase and methionine biosynthesis. The results with these two mutants support the hypothesis that there is a common regulatory element(s) for these enzymes. This led us to examine carefully the effects of previously reported mutations in the *metJ* and *metK* genes causing resistance to methionine analogues and altered regulation of the methionine biosynthetic enzymes. Mutations in the *metJ* gene reportedly affect a repressor for the methionine pathway (1, 5, 9–12), whereas the *metK* gene product is presumably *S*-adenosylmethionine synthetase (1, 5, 9, 10, 12). Because the two strains we isolated appeared similar to other *metK* mutants, we examined this gene-enzyme relationship more carefully by isolating and characterizing a mutant in which the *S*-adenosylmethionine synthetase has an increased apparent K_m for methionine. The results with this mutant support the idea that the *metK* locus codes for *S*-adenosylmethionine synthetase and that either the enzyme itself or its product is involved during repression of both the glycine and methionine biosynthetic enzymes.

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MATERIALS AND METHODS

Strains and media. The bacterial strains used are listed in Table 1. Transductions were performed with KB1 *int-1* phage (3, 14). Both the glucose minimal medium and the Luria broth complex medium have been described (4). Supplements were added at the following concentrations, except where otherwise indicated: amino acids, 200 μg/ml (serine was routinely added at 400 μg/ml); purines and pyrimidines, 10 μg/ml.

Growth. Cultures were grown overnight at 37°C in 0.02% glucose minimal medium with the appropriate supplements and used to inoculate the 0.4%

TABLE 1. *List of bacterial strains used in this investigation*

Strain	Genotype	Relevant phenotype	Source
JL781	+	Wild type	J. Ingraham
JB507	*serA13*	Requires L-serine or glycine	K. Sanderson
JB561	*serA13 glyA951*	Requires L-serine	This lab
JB564	*serA13 glyA20*	Requires L-serine and glycine	This lab
JB567	*glyA951*	Partial requirements for glycine and L-methionine	This lab
JB598	HfrB2 *metK*^{x}*725*	Resistant to methionine analogues[a]	A. Hobson
JB611	+	Wild type	Transductant
JB612	*metJ*	Resistant to ethionine	Transductant
JB613	+	Wild type	Transductant
JB614	*metK*	Resistant to methionine analogues	Transductant
JB616	*glyA951 metK2052*	Resistant to methionine analogues	This lab
JB640	*glyA951 metK2053*	Resistant to methionine analogues	This lab
JB671	*metK*	Resistant to methionine analogues	D. Lawrence
JB672	*metJ*	Resistant to ethionine	D. Lawrence
JB727	*metK2061*	Resistant to methionine analogues	This lab
JB730	+	Wild type	Transductant
JB731	*metK2061*	Resistant to methionine analogues	Transductant

[a] Methionine analogues are ethionine, α-methylmethionine, and norleucine.

glucose minimal growth medium. The inoculum was added to give a reading of 5 Klett units, and growth was monitored with a Klett-Summerson spectrophotometer using a no. 42 filter.

Cell extracts. A 40-ml volume of cells at a Klett reading of 100 ± 5 units was chilled, and the cells were pelleted by centrifugation at 12,000 × *g* and washed two times in cold 0.85% NaCl. These cells were stored as frozen pellets and used within 24 h. The enzyme activities were stable under these conditions. The thawed cells were resuspended in 0.8 ml of the buffer appropriate for each enzyme assay: 1.0 M phosphate buffer (pH 7.5) for serine transhydroxymethylase; 1.0 M phosphate buffer (pH 7.3) for β-cystathionase; or a 1.5 M Trizma-hydrochloride buffer (pH 8.5) for *S*-adenosylmethionine synthetase. Extracts were prepared by sonifying three times for 15 s with a microprobe on a Bronwell Biosonik IV sonicator at a 50% setting. Debris was removed by centrifugation of the extract at 17,300 × *g* for 20 min, and the extracts were used within 1 h for enzyme assays. All results are the averages of duplicate or triplicate determinations with each extract, and each experiment was done at least two times.

Enzyme assays. Serine transhydroxymethylase activity was determined by the procedure of Scrimgeour and Huennekens (25) with slight modification (27). β-Cystathionase activity was determined as previously described, with the exception that L-cystathionine was used to start the reaction (26). *S*-adenosylmethionine synthetase activity was assayed by a modified method of Holloway et al. (10). The complete reaction mixture contained, in 1.0 ml: Trizma-hydrochloride (pH 8.5), 150 μmol; KCl, 100 μmol; $MgCl_2$, 15 μmol; glutathione, 8 μmol; L-methionine, 20 μmol; [^{14}C]adenosine 5′-triphosphate, 10 μmol (0.25 μCi). After 45 min at 37°C, 1.0 ml of 6% perchloric acid was added to stop the reaction. This was followed by centrifugation for 5 min to remove protein, after which a 1.0-ml sample of each supernatant fluid was pipetted onto a column (0.5 by 4.5 cm) of Dowex-50W (hydrogen form; 200 to 400 mesh). Each column was washed with three 10-ml samples of 1.0 N HCl, followed by 5 ml of water to remove residual HCl. A 9-ml volume of 1.0 N NH_4OH was then added to each column to elute the [^{14}C]*S*-adenosylmethionine formed. The eluates were collected and acidified, and a 2-ml sample was counted in Hydromix liquid scintillation counting cocktail.

Protein determination. Protein was determined by the method of Lowry et al. (13), with bovine serum albumin used as the standard. Trizma-hydrochloride was added to the samples for the standard curve when the extracts to be assayed for *S*-adenosylmethionine synthetase activity were prepared in Trizma-hydrochloride (22).

Chemicals. DL-Ethionine, DL-norleucine, and α-methyl-DL-methionine were obtained from Sigma Chemical Co. (St. Louis, Mo.). [^{14}C]adenosine 5′-triphosphate came from Schwarz/Mann (Orangeburg, N.Y.). All other chemicals were reagent grade and commercially available.

RESULTS

Isolation of mutants with altered serine transhydroxymethylase regulation. We wanted to determine whether mutants selected for derepressed levels of serine transhydroxymethylase would have altered regulation specifically for this enzyme or for both serine transhydroxymethylase and enzymes of the methionine biosynthetic pathway. For this selection, advantage was taken of the phenotype of strains carrying the *glyA951* mutation, which reduces the enzyme activity to about 44% of the level found in the wild-type strain. When a strain carrying the *glyA951* lesion is inoculated into a medium supplemented with serine,

methionine, adenine, guanine, and thymine, the enzyme level is repressed and growth ceases due to an inability of the strain to produce sufficient glycine (27). Thus, strain JB567 was plated on minimal medium supplemented with serine, methionine, adenine, guanine, and thymine (but no glycine) to select mutants with an altered or nonrepressible serine transhydroxymethylase. Several mutants were selected by this procedure. Two strains, JB616 and JB640, are of particular interest because they simultaneously became resistant to growth inhibition by the five compounds and resistant to the methionine analogues ethionine, norleucine, and α-methylmethionine. Mutants resistant to these analogues have generally been classified as *metK* mutants. Thus, the new mutation in strain JB616 was tentatively designated *metK2052*, and the new mutation in strain JB640 was tentatively designated *metK2053*.

Serine transhydroxymethylase levels in strains JB616 and JB640. Since the procedure used to isolate strains JB616 and JB640 presumably selects for mutants with altered regulation of serine transhydroxymethylase, the activity of this enzyme was measured in the mutant strains (Table 2). Strain JL781 (wild type) had a specific activity of 192 nmol of HCHO consumed/mg of protein per min, which was reduced threefold when this strain was grown in the presence of serine, glycine, methionine, adenine, guanine, and thymine. Strain JB567 in unsupplemented medium showed the expected reduced activity caused by the *glyA951* mutation; however, the enzyme levels in strains JB616 and JB640 are 1.5- and 2.5-fold higher, respectively. Furthermore, when the mutants were grown in the presence of the six compounds, the serine transhydroxymethylase levels were only slightly reduced in JB616 and JB640. Although these differences are small, they are reproducible and explain the ability of these two strains to grow on the selection plates.

Genetic characterization of strains JB616 and JB640. Transductions were performed to determine whether strains JB616 and JB640 retained the original *glyA951* mutation. KB1 *int-1* phage grown on strains JL781 (*glyA*$^+$), JB561 (*serA13 glyA951*), JB616 (*glyA951 metK2052*), and JB640 (*glyA951 metK2053*) were used in transductions with strain JB564 (*serA13 glyA20*) as the recipient on serine-supplemented plates. Strain JB561 (*serA13 glyA951*) requires only serine for growth and cannot use glycine as a serine source, whereas strain JB564 (*serA13 glyA20*) requires both serine and glycine for growth (27). Transductants receiving either wild-type deoxyribonucleic acid or *glyA951* deoxyribonucleic acid for the *glyA* region should grow on the selective medium with serine but could be distinguished from each other by their ability or inability to grow in medium supplemented with glycine. All the transductants with strain JL781 as donor were able to use either serine or glycine to fulfill the serine requirement, showing that wild-type deoxyribonucleic acid replaced *glyA20* (see Table 3). However, with strain JB561 as donor, all of the transductants were unable to use glycine as a serine source, demonstrating the replacement of *glyA20* with the *glyA951* lesion. This same result was found for transductants with strain JB616 (*glyA951 metK2052*) and strain JB640 (*glyA951 metK2053*) as donors. Thus, strains JB616 and JB640 both carry the *glyA951* mutation, and their new phenotypes result from second mutations not closely linked to the *glyA* gene.

The resistance of strains JB616 and JB640 to the methionine analogues suggested that the new mutation could be in either the *metJ* or

TABLE 2. *Results of enzyme assays for strains JL781, JB567, JB616, and JB640 grown in the presence of the indicated supplements*

Additions to glucose minimal medium	Strain	Enzyme activities[a]	
		Serine transhydroxymethylase	β-Cystathionase
None	JL781	192 (100)	6.36 (100)
Serine + glycine + methionine + adenine + guanine + thymine	JL781	67 (35)	2.21 (35)
None	JB567	81 (42)	13.75 (216)
Serine + glycine + methionine + adenine + guanine + thymine	JB567	36 (19)	2.89 (45)
None	JB616	124 (65)	7.66 (120)
Serine + glycine + methionine + adenine + guanine + thymine	JB616	79 (41)	2.42 (38)
None	JB640	191 (100)	7.29 (115)
Serine + glycine + methionine + adenine + guanine + thymine	JB640	138 (72)	1.63 (26)

[a] Values are given as specific activities (nanomoles of product/milligram of protein per minute) and percentage of wild-type (JL781) activity in glucose minimal medium (numbers in parentheses). Concentrations of supplements are as stated in Materials and Methods. The results are the averages of at least five separate experiments for serine transhydroxymethylase levels and three experiments for the β-cystathionase levels.

TABLE 3. *Results of transductions with revertants of strains carrying the glyA951 lesion*[a]

Donor[b]	Recipient	No. of colonies growing on scoring medium		
		Minimal	Glycine	Serine
JL781 *glyA*$^+$	JB564 *serA13 glyA20*	0	94	94
JB561 *serA13 glyA951*	JB564 *serA13 glyA20*	0	0	86
JB616 *glyA951 metK2052*	JB564 *serA13 glyA20*	0	0	34
JB640 *glyA951 metK2053*	JB564 *serA13 glyA20*	0	0	32

[a] All transductants were selected on medium supplemented with serine and then spotted on the scoring media.
[b] The presumed genotype for the donor strains JB616 and JB640 is given for convenience. This experiment was to determine if the *glyA951* mutation is present.

metK gene, which influence the regulation of the methionine pathway. To examine this possibility, transducing phage were grown on strains JL781 (wild type) JB671 (*metK*), JB672 (*metJ*), JB616 (*glyA951 metK2052*), and JB640 (*glyA951 metK2053*) and used to transduce strain JB507 (*serA13*) to serine independence, or strain JB516 (*metB63*) to methionine independence. Transductants were then scored for analogue resistance (Table 4). Strains JB671 and JB672 were included as controls to verify the validity of the selection and scoring techniques. Strain JB671 is a *metK* mutant, in which the mutant allele contransduced with *serA13* at a frequency of 2.4%, whereas strain JB672 is a *metJ* mutant, in which the mutant allele contransduced with *metB63* at a frequency of 84.2%. With strain JB616 as the donor, the ethionine resistance cotransduced with the *serA13* lesion at a frequency of 11%, and with strain JB640, the ethionine resistance cotransduced with *serA13* at a frequency of 1.8%. These results (Table 4) show that the mutations leading to analogue resistance in strains JB616 and JB640 are linked to the *serA* gene, as is the control *metK* mutation, and thus substantiate the tentative designation of these strains as *metK* mutants.

S-adenosylmethionine synthetase levels in strains JB616 and JB640. Other *metK* mutants have been reported to have reduced S-adenosylmethionine synthetase activity. Therefore, strains JB598 (*metK*^{ts}*725*), JB567 (*glyA951*), JB616 (*glyA951 metK2052*), and JB640 (*glyA951 metK2053*) were grown in glucose minimal medium (with and without a methionine supplement), and extracts were assayed for S-adenosylmethionine synthetase activity (Table 5). The specific activity for strain JB567 was set at 100% activity; the addition of methionine to the growth medium reduced this to 24% of the level found in the unsupplemented medium. The S-adenosylmethionine synthetase levels are slightly reduced in both JB616 and JB640. Growth in the presence of methionine repressed the levels to approximately the same extent in all strains (Table 5). As previously reported, strain JB598 (*metK*^{ts}*725*), obtained from A. Hobson, has less than 8% of the activity found in the control strain JB567.

β-Cystathionase levels in strains JB616 and JB640. Because some *metK* mutants have slightly increased and nonrepressible levels of

TABLE 4. *Results of transductions with strains JL781, JB671, JB672, JB616, and JB640*[a]

Donor	Recipient	No. of colonies growing on scoring medium		Cotransduction (%)[b]
		Ethionine[c]	Minimal	
JL781 wild type	JB507 *serA13*	0	268	0.0
JL781 wild type	JB516 *metB63*	0	76	0.0
JB671 *metK*	JB507 *serA13*	9	380	2.4
JB672 *metJ*	JB516 *metB63*	64	76	84.2
JB616 *metK2052*	JB507 *serA13*	88	798	11.0
JB616 *metK2052*	JB516 *metB63*	0	76	0.0
JB640 *metK2053*	JB507 *serA13*	9	494	1.8
JB640 *metK2053*	JB516 *metB63*	0	76	0.0

[a] All transductants were selected on medium supplemented with serine and then spotted on the scoring media.
[b] Percent cotransduction = (number of transductants resistant to ethionine/total number of transductants) × 100.
[c] Concentration of ethionine is 1.0 mg/ml.

TABLE 5. *S-adenosylmethionine synthetase activity for strains JB567, JB598, JB616, and JB640 grown in the presence of the indicated supplements*

Additions to glucose minimal medium	Strain	Enzyme activity[a]
None	JB567 *glyA951*	9.5 (100)
Methionine	JB567 *glyA951*	2.3 (24)
None	JB616 *glyA951 metK2052*	5.8 (61)
Methionine	JB616 *glyA951 metK2052*	1.8 (19)
None	JB640 *glyA951 metK2053*	4.5 (47)
Methionine	JB640 *glyA951 metK2053*	1.5 (16)
None	JB598 *metK*^{ts}*725*	0.4 (4)
Methionine	JB598 *metK*^{ts}*725*	0.8 (8)

[a] Values are given as specific activities (nanomoles of S-adenosylmethionine formed/milligram of protein per minute) and percentage of strain JB567 activity when grown in glucose minimal medium (numbers in parentheses). Concentration of methionine is 200 μg/ml. The results are the averages of two separate experiments, with a maximum of 10% variation from the reported average.

the methionine biosynthetic enzymes, β-cystathionase levels were also measured in these strains as an indication of the regulation of the methionine pathway. Strain JB567 (*glyA951*) had a twofold higher level of β-cystathionase than strain JL781 (wild type), whereas the levels in strains JB616 (*glyA951 metK2052*) and JB640 (*glyA951 metK2053*) were only slightly higher than those found in strain JL781 (all grown in unsupplemented media) (Table 2). Thus, the β-cystathionase levels are increased slightly in strains JB616 and JB640 compared with the wild-type strain JL781, but the new mutations in these strains result in a decrease in enzyme levels compared with their parent strain JB567. Finally, addition of the six compounds to the growth medium repressed β-cystathionase to the same level in all strains.

Serine transhydroxymethylase levels of *metK* and *metJ* mutants. It has been reported that *metK* and *metJ* mutants of *E. coli* have altered regulation of serine transhydroxymethylase (6, 16). These reports, coupled with the results presented above for strains JB616 and JB640, suggest that the serine transhydroxymethylase reaction shares some common regulatory element(s) with the methionine pathway. To further examine this question in *S. typhimurium*, *metK* and *metJ* mutants were examined for altered serine transhydroxymethylase levels. To insure that the effects of serine transhydroxymethylase were due to lesions in either the *metJ* or *metK* gene and not to other differences in the mutants, isogenic strains for each mutation were constructed. From a transduction of strain JB671 (*metK*) into the recipient JB507 (*serA13*), one ethionine-sensitive (JB613, *metK*$^+$) and one ethionine-resistant (JB614, *metK*) transductant were isolated. These strains are isogenic except for the *metK* region of the chromosome. The same procedure was used with strain JB672 (*metJ*) into the recipient strain JB516 (*metB63*). An ethionine-sensitive transductant was designated JB611 (*metJ*$^+$) and an ethionine-resistant transductant was designated JB612 (*metJ*). These strains are isogenic except for the *metJ* region of the chromosome.

The serine transhydroxymethylase levels for these strains grown in minimal medium or minimal medium supplemented with excess serine, glycine, methionine, adenine, guanine, and thymine are given in Table 6. The addition of these compounds to the growth medium reduced the serine transhydroxymethylase activity in strain JB613 (*metK*$^+$) to 12% of the level found in unsupplemented medium. Conversely, the levels in strain JB614 (*metK*$^-$) were not significantly decreased in cells grown in the supplemented medium. Similar results were found with the *metJ* mutant. Strain JB612 (*metJ*$^-$) had 81% of the enzyme activity found in the *metJ*$^+$ strain when grown in unsupplemented medium, and growth in the six compounds lowered the level only slightly. Thus, serine transhydroxymethylase regulation is altered in both *metK* and *metJ* mutants, and, apparently, a common regulatory element for the methionine and serine/glycine pathways exists in *S. typhimurium*. The reason for the difference in the total specific activity for the two control strains, JB613 and JB611, is unknown, but the results demonstrate the importance of constructing isogenic strains when comparing enzyme levels.

TABLE 6. *Serine transhydroxymethylase activity for strains JB611, JB612, JB613, and JB614 grown in the presence of the indicated supplements*

Additions to glucose minimal medium	Strain	Enzyme activity[a]
None	JB613 *metK*$^+$	95 (100)
Serine + glycine + methionine + adenine + guanine + thymine	JB613 *metK*$^+$	11 (12)
None	JB614 *metK*$^-$	78 (82)
Serine + glycine + methionine + adenine + guanine + thymine	JB614 *metK*$^-$	80 (84)
None	JB611 *metJ*$^+$	176 (100)
Serine + glycine + methionine + adenine + guanine + thymine	JB611 *metJ*$^+$	72 (41)
None	JB612 *metJ*$^-$	143 (81)
Serine + glycine + methionine + adenine + guanine + thymine	JB612 *metJ*$^-$	131 (74)

[a] Values are given as specific activities (nanomoles of HCHO consumed/milligram of protein per minute) or as percentage of control (the percentage of strain JB613 activity when grown in glucose minimal medium for the *metK* strains, and the percentage of strain JB611 activity when grown in glucose minimal medium for the *metJ* strains) (numbers in parentheses). Concentrations of supplements are as stated in Materials and Methods. The results are the average of six separate experiments.

Gene-enzyme relationship for the *metK* locus. Although there is a correspondence between the analogue-resistant phenotype of the *metK* mutants and reduced levels of *S*-adenosylmethionine synthetase, it is not clear whether the *metK* gene actually encodes this enzyme in *S. typhimurium*. The possibility exists that the altered regulation of the methionine biosynthetic enzymes and reduced *S*-adenosylmethionine synthetase activity in the *metK* mutants could be caused indirectly by another component distinct from *S*-adenosylmethionine synthetase itself. To examine the gene-enzyme relationship for *S*-adenosylmethionine synthetase, an attempt was made to isolate a mutant with an altered, rather than a reduced, *S*-aden-

osylmethionine synthetase activity.

Several compounds were tested to determine whether or not they inhibit *S*-adenosylmethionine synthetase activity in vitro. At 10 mM the following compounds had no effect on enzyme activity: DL-ethionine, DL-norleucine, thialysine, putrescine, and spermidine. One compound, α-methyl-DL-methionine, at 5 mM inhibited more than 50% of the *S*-adenosylmethionine synthetase activity. Although it has been reported previously that α-methylmethionine inhibits only homoserine *O*-transsuccinylase activity (*metA* gene product; 12, 23, 24), these results show that *S*-adenosylmethionine synthetase is also sensitive. It was thought possible, therefore, that mutants resistant to this analogue would have an *S*-adenosylmethionine synthetase activity resistant to α-methylmethionine. Thus, 10 mutants resistant to α-methylmethionine were selected from strain JL781 and characterized for their resistance to other analogues and their *S*-adenosylmethionine synthetase activities. Nine of the mutants had simultaneously become norleucine and ethionine resistant. Some also lost *S*-adenosylmethionine synthetase activity and resembled the *metK* mutants described earlier. However, in one strain (JB727) the activity was only slightly reduced, from 13.3 to 8.6 units, and was of particular interest because the remaining activity was resistant to inhibition by α-methylmethionine.

To further examine the inability of α-methylmethionine to inhibit the *S*-adenosylmethionine synthetase activity in this strain, a more detailed inhibition study was performed using varying concentrations of α-methylmethionine. A concentration of 4 mM α-methyl-DL-methionine is required for 50% inhibition in strain JL781, whereas a concentration of 22 mM is required for 50% inhibition in strain JB727 (Fig. 2). This difference was substantiated by comparing the activities with increasing methionine concentrations added to the reaction mixture. The apparent K_m and V_{max} values determined from plots of $1/v$ versus $1/[s]$ show that the apparent K_m for methionine is increased 26-fold in strain JB727 compared to the apparent K_m of strain JL781 (0.15 mM methionine versus 3.80 mM methionine). The V_{max} was slightly lower in the mutant strain JB727 (6.8 compared to 8.7 for JL781). These results show a direct alteration of the *S*-adenosylmethionine synthetase, suggesting that this mutation lies in the structural gene for *S*-adenosylmethionine synthetase.

To determine whether or not this mutation conferring analogue resistance lies in the *metK* gene, a transduction experiment was performed (Table 7). Transducing phage were grown on strains JL781 and JB727 and used to transduce strain JB507 (*serA13*) to serine independence. With strain JL781 (*metK*$^+$) as the donor, no analogue-resistant transductants were found. When strain JB727 (*metK2061*) was the donor, 2.8% of the transductants were analogue resistant (comparable to the frequency found with the control *metK* strain shown in Table 7). This again suggests the new

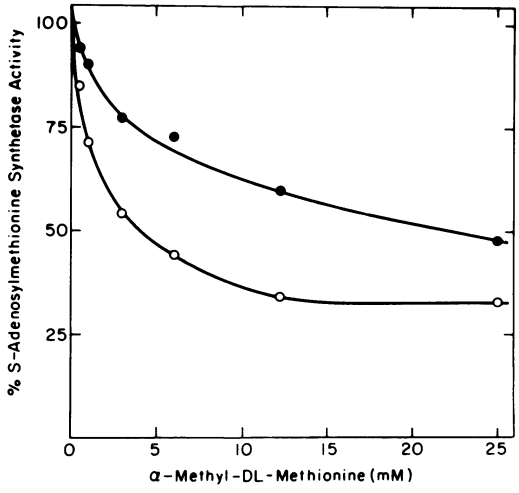

FIG. 2. *Inhibition of S-adenosylmethionine synthetase activity by α-methyl-DL-methionine. Cell extracts of strain JL781 (wild type) and strain JB727 (metK2061) grown in glucose minimal medium were assayed for S-adenosylmethionine synthetase activity in the presence of α-methyl-DL-methionine at concentrations between 0.0 and 25.0 mM. The 100% activity is equivalent to a specific activity of 9.8 nmol of S-adenosylmethionine formed/mg of protein per min for strain JL781 (○) and 6.4 nmol of S-adenosylmethionine formed/mg of protein per min for strain JB727 (●).*

TABLE 7. *Results of transductions with strains JL781, JB671, and JB727*[a]

Donor	Recipient	No. of colonies growing on scoring medium			Cotransduction (%)[b]
		Minimal	Ethionine	α-Methyl-DL-methionine[c]	
JL781 *metK*$^+$	JB507 *serA13*	384	0	0	0.0
JB671 *metK*$^-$	JB507 *serA13*	380	9	9	2.4
JB727 *metK2061*	JB507 *serA13*	612	17	17	2.8

[a] All transductants were selected on glucose minimal medium and then spotted on the scoring media.

[b] Percent cotransduction = (number of transductants resistant to analogues/total number of transductants scored) × 100.

[c] Concentrations of analogues are 1 mg/ml.

mutation in strain JB727 lies in, or close to, the *metK* gene, and that the *metK* gene encodes the enzyme *S*-adenosylmethionine synthetase.

Properties of a *metK2061* strain. One analogue-sensitive (*metK*$^+$) and one analogue-resistant (*metK2061*) transductant from a transduction with strain JB727 as donor were saved and designated strains JB730 and JB731, respectively. Results of *S*-adenosylmethionine synthetase assays with extracts prepared from these strains were almost identical to the results presented for strains JL781 (*metK*$^+$) and JB727 (*metK2061*) (data not shown), indicating that all the properties of the original mutant strain JB727 are due to a single mutation. Since the parent strain was not mutagenized, it is likely that a single mutation is present in strains JB727 and JB731.

The *S. typhimurium metK* mutants that have been examined in detail have been divided into two classes (7, 9, 11, 12). The mutants of one class excrete methionine, show no measurable *S*-adenosylmethionine synthetase activity, and contain high levels of methionine biosynthetic enzymes that are nonrepressible by methionine. The second class does not excrete methionine, shows low *S*-adenosylmethionine synthetase activity, and contains methionine biosynthetic enzymes that are repressed by methionine. Measurements of β-cystathionase activity showed that the enzyme level was higher in strain JB731 (*metK2061*) than in strain JB730 (*metK*$^+$) for minimal medium-grown cells (24.9 versus 16.1 nmol of homocysteine/mg of protein per min) and that this enzyme is repressed in both strains for methionine-grown cells (5.1 and 3.0 nmol of homocysteine/mg of protein per min, respectively). Because strain JB731 does not excrete methionine and β-cystathionase synthesis is repressed by a methionine supplement to the growth medium, strain JB731 is typical of the second class of *metK* mutants.

DISCUSSION

This work has examined the relationship between serine transhydroxymethylase activity and its function in glycine and methionine synthesis in an attempt to identify components of the regulatory mechanism for this enzyme. Strains JB616 and JB640 were isolated using a procedure designed to select mutants with either altered levels or regulation of serine transhydroxymethylase. Both strains have elevated levels of serine transhydroxymethylase relative to the parent strain JB567, and synthesis of this enzyme is not as sensitive to repression as it is in either the parent strain JB567 or the wild-type strain JL781 (Table 2). In addition, both strains have several properties characteristic of *metK* mutants. They are resistant to the methionine analogues ethionine, norleucine, and α-methylmethionine, the mutation in each strain that confers analogue resistance cotransduces at a low frequency with a *serA* marker (Table 4), and both strains have a reduced *S*-adenosylmethionine synthetase activity (Table 5). Thus, by a procedure that selects for mutants having altered levels of serine transhydroxymethylase, two mutants with the *metK* phenotype were isolated. In addition, an examination of other strains carrying *metK* or *metJ* mutations show similar nonrepressible enzyme levels. These data support the hypothesis that the glycine and the methionine pathways share a common regulatory element.

It is interesting that β-cystathionase levels are higher in strain JB567 (*glyA951*) than in strains JB616 (*glyA951 metK2052*) and JB640 (*glyA951 metK2053*) (Table 2). Presumably the *glyA951* lesion in strain JB567 causes a partial starvation of methionine (and *S*-adenosylmethionine), leading to elevated levels of the methionine biosynthetic enzymes. This is supported by the fact that the addition of methionine to glucose minimal medium reduces the generation time of strain JB567 from 97 to 76 min. Strains JB616 and JB640 have generation times of 70 and 78 min, respectively, in glucose minimal medium. This is possibly due to the increased serine transhydroxymethylase levels in these strains due to the *metK2052* and *metK2053* lesions (Table 2), which in turn allows more one-carbon units to be generated for increased methionine production (Fig. 1).

These results clarify some aspects of serine transhydroxymethylase regulation. First, some mutants selected for increased serine transhydroxymethylase expression are also affected in the regulation of the methionine biosynthetic pathway. Second, mutations (*metK*) affecting *S*-adenosylmethionine synthetase activity prevent complete repression of serine transhydroxymethylase. Third, mutations (*metJ*) possibly affecting the repressor for the methionine biosynthetic enzymes also eliminate full repression of serine transhydroxymethylase levels. These results are qualitatively consistent with others reported for *E. coli* mutants carrying lesions in either the *metJ* or *metK* gene (6, 16).

The combination of these results establishes that serine transhydroxymethylase is partially controlled as a methionine biosynthetic enzyme; however, there are some unique features. For example, the *metJ* mutants have increased levels of the methionine enzymes in both minimal and supplemented media. The

serine transhydroxymethylase activity, however, is not increased in cells grown in minimal medium, but the levels cannot be repressed in cells grown in supplemented medium (Tables 2 and 6). If the *metJ* product is the sole repressor for both pathways, an increase in serine transhydroxymethylase levels would be expected. The non-repressibility of this enzyme in a *metJ* mutant (Table 6) indicates that this protein is necessary as a component of the repression process, but its absence does not lead to derepression.

It has been proposed that the *metJ* gene product interacts with *S*-adenosylmethionine to cause repression of the methionine biosynthetic enzymes (5, 9). Our results are generally consistent with these two components also being important for serine transhydroxymethylase control. For example, three growth conditions may cause increased serine transhydroxymethylase levels: (i) growth limitation for methionine (6, 15); (ii) growth limitation for purines (26); and (iii) growth with the folate inhibitor trimethoprim (26). All three of these could indirectly cause a reduction in the *S*-adenosylmethionine pool. Furthermore, both our results with the *metK* mutants of *S. typhimurium* and those with *E. coli* (6, 16) suggest that *S*-adenosylmethionine production is important in this regulatory mechanism.

Unfortunately, information derived from *metK* mutants is complicated. Although there is substantial evidence that mutations in the *metK* gene affect the *S*-adenosylmethionine synthetase levels, it is not clear how the production of *S*-adenosylmethionine is affected. Mutants lacking essentially all *S*-adenosylmethionine synthetase activity grow well and maintain their *S*-adenosylmethionine pool (7–10), and Hobson (8) has suggested that a second route to *S*-adenosylmethionine exists. If this is true, then results with *metK* mutants must be interpreted carefully to distinguish between an altered gene product (*S*-adenosylmethionine synthetase), which itself may affect regulation, versus a depletion of an enzyme product (*S*-adenosylmethionine), which is needed for regulation.

Because of these and other problems in understanding results with *metK* mutants, we felt it was important to examine whether mutations causing this phenotype were actually in the *S*-adenosylmethionine synthetase structural gene or in another gene that only affected levels of the enzyme. Thus, we selected an α-methylmethionine-resistant mutant in which the *S*-adenosylmethionine synthetase activity is less sensitive to this inhibitor. Further characterization demonstrated that this enzyme has a reduced affinity for methionine and the strain has a phenotype associated with other *metK* mutants. Thus, it appears that lesions in the *metK* gene do affect the *S*-adenosylmethionine enzyme directly. Mutant JB727 with the altered *S*-adenosylmethionine synthetase should be useful in future experiments designed to determine whether two pathways to *S*-adenosylmethionine exist and whether it is *S*-adenosylmethionine or *S*-adenosylmethionine synthetase that is the key component for methionine and/or serine transhydroxymethylase regulation.

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