Regulation of S-Adenosylmethionine Synthetase in Escherichia coli

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Addition of methionine to the growth medium of *Escherichia coli* K-12 leads to a reduction in the specific activity of S-adenosylmethionine (SAM) synthetase. Thus the enzyme appears to be repressible rather than inducible. Mutant strains (probably metJ⁻) are constitutive for SAM synthetase as well as for the methionine biosynthetic enzymes, suggesting that the regulatory systems for these enzymes have at least some elements in common. Cells grown to stationary phase in complete medium, which have low specific activities of the enzymes, were routinely used for derepression experiments. The lag in growth and derepression when these cells are incubated in minimal medium is shortened by threonine. Ethionine, norleucine, and α-methylmethionine are poor substrates or nonsubstrates for SAM synthetase and are ineffective repressors. Selenomethionine, a better substrate for SAM synthetase than methionine, is also slightly more effective at repression than methionine. Although SAM is considered to be a likely candidate for the corepressor in the control of the methionine biosynthetic enzymes, addition of SAM to the growth medium does not cause repression. Measurement of SAM uptake shows that too little is taken into the cells to have a significant effect, even if it were active in the control system.

The control of the biosynthesis of the aspartic family of amino acids in *Escherichia coli* has been studied by many workers. This system, which has served as a model for the control of branched biosynthetic pathways, has been the subject of several recent reviews (3, 31, 32). The enzymes of this pathway which are unique to methionine biosynthesis are repressed by addition of methionine to the growth medium (16, 23, 24, 30, 33). The structural genes for these enzymes are scattered in the genetic map of *E. coli* but, since apparently single gene mutations lead to loss of ability to repress any of them, they all seem to be controlled by a single regulatory system (2, 13, 16, 22). None of these reports considered control of S-adenosylmethionine (SAM) synthetase. We have found that SAM synthetase is repressed by addition of methionine to the growth medium and have isolated ethionine-resistant mutants of *E. coli* K-12 which are constitutive for SAM synthetase, cystathionine synthetase, and cystathionase. The results suggest that control of SAM synthetase shares at least some regulatory elements with that of the methionine biosynthetic enzymes.

MATERIALS AND METHODS

Materials. Glutathione, disodium adenosine triphosphate (ATP), and chloramphenicol were obtained from the Sigma Chemical Co.; L-threonine, L-norleucine, and L-cystathionine were from Calbiochem; DL-, D-, and L-methionines and L-ethionine were from Mann Research Laboratories; DL-α-methylmethionine and DL-selenomethionine were from the Cyclo Chemical Corp.; reduced nicotinamide adenine dinucleotide (NADH) was from Pabst Laboratories, 5,5' dithio-bis (2-nitrobenzoic acid) from the Aldrich Chemical Co., and beef heart lactic dehydrogenase from the Worthington Biochemical Corp. Disodium ATP-8-¹⁴C was obtained from Schwarz BioResearch Inc. Unlabeled SAM was prepared by a modification of the method of Schlenk et al. (25) by using chromatographic purification similar to that of Shapiro and Ehninger (28). Radioactive SAM was prepared by incubating ATP-8-¹⁴C and methionine with partially purified yeast SAM synthetase (8) followed by chromatography on Dowex 50 X-8 (minus 400 mesh) with a gradient from 0 to 6 N HCl. The radioactive SAM peak fractions were combined and lyophilized. The unlabeled SAM gave a single ultraviolet-absorbing, ninhydrin-reactive spot on paper electrophoresis. Between 95 and 98% of the radioactivity of the labeled SAM migrated with the unlabeled SAM. Degradation
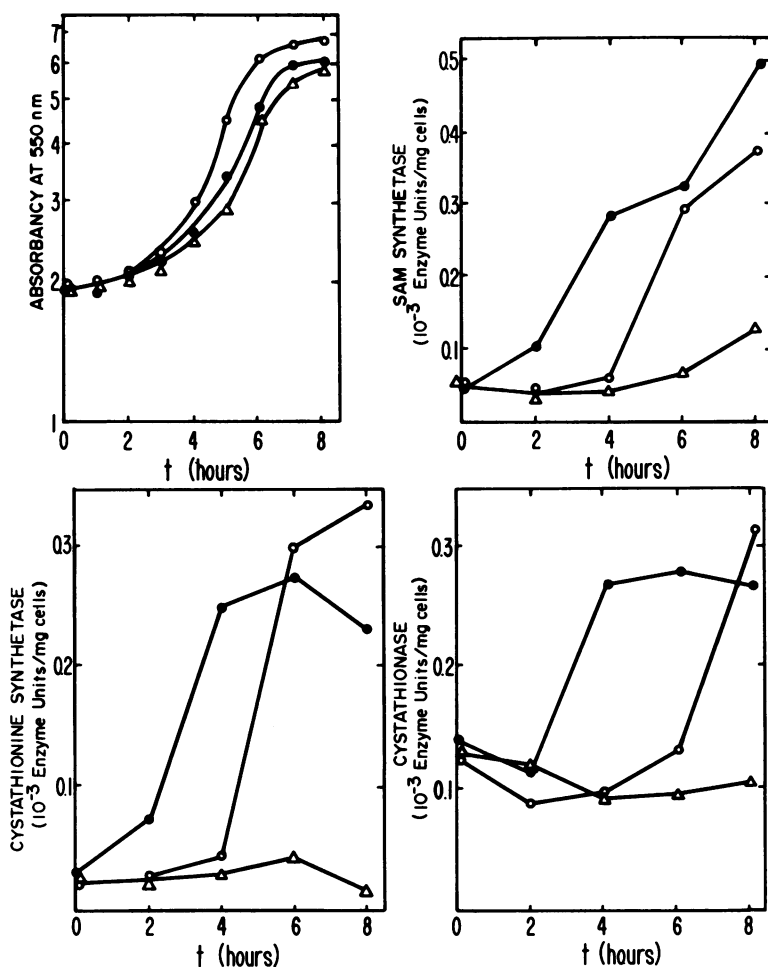
FIG. 1. *Effect of methionine on derepression of wild-type cells. Wild-type cells were grown in LB broth, harvested, and incubated in minimal dextrose medium containing (●) no methionine, (○) 2 × 10⁻⁴ M L-methionine or (△) 10⁻³ M L-methionine.*

products which accumulate on long-term storage can be removed by rechromatography on Dowex 50 X-2 by using 1 N HCl to wash off impurities and by eluting the SAM with 3 N HCl.

O-succinylhomoserine was synthesized by the method of Flavin and Slaughter (7). The product had the reported melting point and infrared spectrum.

Bacterial strains. Wild-type *E. coli* K-12 (λ)F+ was obtained from Samson Gross of the Biochemistry Department at Duke University. Several strains which are constitutive for SAM synthetase (exemplified here by D8 and E31) were isolated as spontaneous ethionine-resistant mutants of wild-type *E. coli* K-12. The genetics of these strains will be reported in detail elsewhere. Transduction experiments have shown them to be closely linked to the *metB* locus (C-H. Su, R. C. Greene, and C. T. Holloway, Bacteriol. Proc., p. 136, 1970), and they are probably mutations at the *metJ* locus described by Lawrence, Smith, and Rowbury (13) in *Salmonella typhimurium*.

Media and growth of cells. Chemically defined media were based on a modification of the medium of Davis and Mingioli (4) containing, per liter: 7 g of K_2HPO_4, 3 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, and 5 g of dextrose (or in some cases 5 g of sodium lactate) with supplementation as required. Complete media were LB broth (27) containing 10 g of tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl per liter, or nutrient broth (Difco). Cells were routinely grown at 37 C and 250 rev/min in a New Brunswick model G-25 gyrotory incubator shaker. For derepression experiments, cells were grown overnight (∼16 hr) in 1-liter Bellco shake flasks containing 500 ml of medium (usually LB broth). An adequate volume of culture was centrifuged at 11,000 rev/min for 15 min in a Sorvall RC-2B refrigerated centrifuge. The medium was discarded, and the cells were suspended in sufficient minimal medium (0.5% dextrose) to give an absorbancy of 2.0 at 550 nm (∼3 mg of cells/ml). Portions of the cell suspension
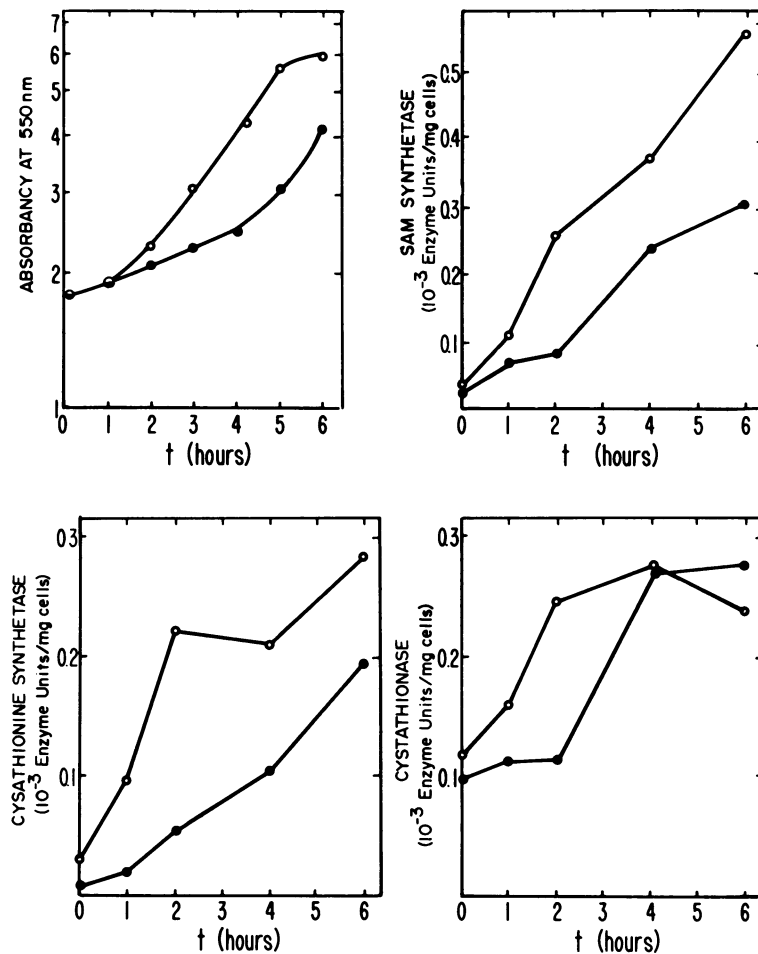
FIG. 2. *Effect of threonine on growth and derepression. Wild-type cells grown to stationary phase in LB broth were incubated in minimal dextrose medium containing* (●) *no threonine or* (○) 5×10^{-3} M *L-threonine.*

(usually 250 ml) were added to 1-liter Bellco shake flasks containing specified supplements. The flasks were shaken to mix the contents and were incubated at 37 C and 150 rev/min in a New Brunswick model G77 Metabolyte shaking water bath. Growth was followed by dilution of 0.5-ml samples of culture to 5 ml with water and measurement of the absorbancy at 550 nm with a Gilford model 240 spectrophotometer. At periodic intervals, samples for enzyme assay were taken as described below.

Harvest and toluenization of cells. Samples of culture fluid were centrifuged for 10 min at 12,000 rev/min in the cold. The supernatant fluid was discarded, and the cells were washed by suspension in 5 ml of 0.02 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 7.6 (3 C), and centrifugation. If the cells were not to be assayed immediately, the pellet was stored in a freezer overnight. The washed cells were suspended in sufficient 0.02 M Tris-hydrochloride, pH 7.6, to give an absorbancy of 20 at 550 nm (calculated from the absorbancy of the culture and the volume of sample taken). Toluene (0.05 volume) was

added, the suspension was incubated at 37 C for 10 min, and the cells were stored in ice until the enzyme assays were completed.

Enzyme assays. Adenosylmethionine synthetase activity was assayed by measuring conversion of ^{14}C-ATP to ^{14}C-SAM with Dowex 50 used to separate the radioactive SAM from other labeled compounds. Assay mixtures contained: 150 μmoles of Tris-hydrochloride, pH 8.5; 100 μmoles of KCl; 15 μmoles of MgCl$_2$; 8 μmoles of glutathione; 20 μmoles of L-methionine; 10 μmoles of ATP-8-^{14}C (0.25 μCi); and 0.4 ml of toluenized cells, in a total volume of 1 ml. The mixtures were incubated for 30 min at 37 C, and the reactions were stopped by the addition of 1 ml of 6% HClO$_4$. The precipitated material was removed by centrifugation and 1 ml of each supernatant fluid was pipetted onto a column (0.6 by 4 cm) of Dowex 50 X-2(H) (200 to 400 mesh). Each column was washed with three 10-ml samples of 1 N HCl followed by 5 ml of water to remove residual HCl and 9 ml of 1 N NH$_3$ to strip off the adenosylmethionine. The NH$_3$ eluates were acidified by the addition of 1 ml of concentrated
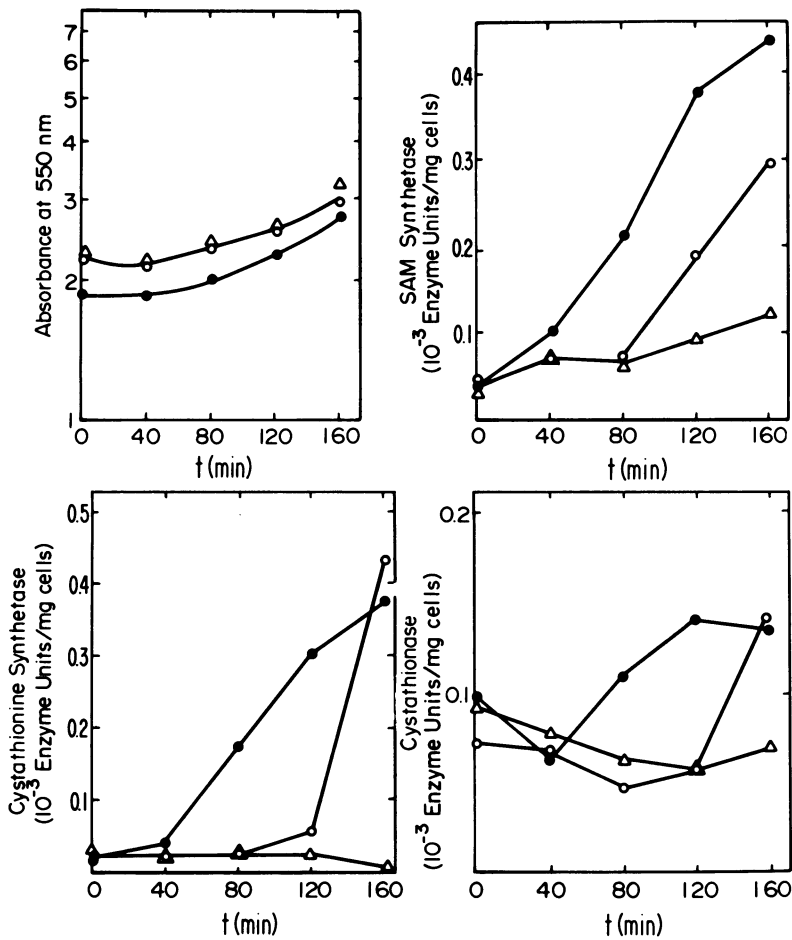
FIG. 3. *Repression of wild-type cells by methionine in the presence of threonine. Cells grown to stationary phase in LB broth were incubated in minimal dextrose medium containing 5 × 10^{-3} M L-threonine and (●) no methionine, (○) 2 × 10^{-5} M L-methionine, or (△) 6 × 10^{-5} M L-methionine.*

HCl and 4-ml samples were counted in the 2:1 toluene Triton X-100 counting mixture of Patterson and Greene (19). A series of incubations with different quantities of toluenized cells gave a slightly curved plot of enzyme activity versus cell concentration. At high rates of SAM synthesis (330 nmoles per incubation mixture per 30 min), specific activities were about 25% lower than those with low rates of SAM synthesis (54 nmoles per incubation mixture per 30 min). In spite of this deviation from linearity, the assay is adequate for comparison of cells with large differences in specific activity. In separate experiments, sonic extracts and toluenized cells gave the same SAM synthetase activity. More than 75% of added labeled SAM could be recovered after incubation with toluenized cells under assay conditions, indicating that extensive degradation did not take place during the assay. The activities of blank incubations without cells or without methionine are comparable and low even when compared to the activities of repressed cells (generally less than 1 nmole per mg of cells). Cystationine synthetase was assayed by a modification of the procedure of

Kaplan and Flavin (11) by measurement of the α-keto-butyrate resulting from the elimination reaction of O-succinylhomoserine. Since at certain stages of the derepression experiments the cells contain a substance that reacts with lactic dehydrogenase and NADH to give high blanks, the toluenized cells were washed by sedimentation and suspension in an equal volume of buffer. Assay mixtures containing: 100 μmoles of Tris-hydrochloride, pH 8.1; 0.125 μmole of pyridoxal phosphate; 5 μmoles of O-succinylhomoserine, and washed toluenized cells (0.2 ml for wild type, 0.1 ml for constitutive mutants) in a total volume of 0.5 ml are incubated for 20 min at 37 C. Incubation tubes are placed in an ice bath, and 0.1 ml of 6% $HClO_4$ is added to each tube. The acid is neutralized by addition of 0.03 ml of 2 M potassium carbonate, and an appropriate volume of supernatant (0.4 ml for wild type, 0.2 ml for constitutive mutants) is added to a cuvette containing 0.2 μmole of NADH and sufficient 0.05 M, pH 7.5, potassium phosphate to give a total volume of 1 ml. The absorbancy of the solution at 340 nm was measured; beef heart lactic dehydrogenase (~25 μg)
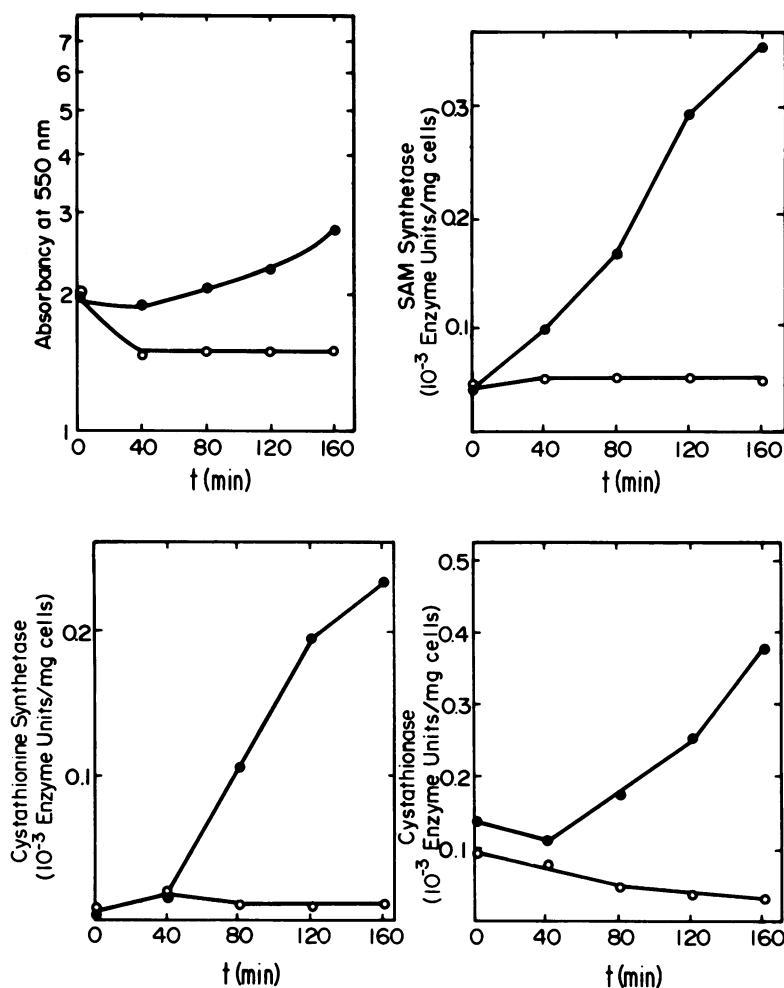
FIG. 4. *Effect of chloramphenicol on enzyme formation. LB broth-grown wild-type cells were incubated in minimal dextrose medium supplemented with 5 × 10⁻³ M L-threonine alone (●), or with the addition of 40 µg/ml of chloramphenicol (○).*

TABLE 1. *Enzyme activities of wild-type and constitutive strains of Escherichia coli[a]*

Strain	Enzyme activities[b]		
	SAM synthetase	Cysta-thionine synthetase	Cysta-thionase
Wild type	0.037	0.015	0.1
E31	0.82	1.83	4.4
D8	0.83	1.88	3.2

[a] Cells were grown to stationary phase in LB broth, harvested, toluenized, and assayed.

[b] Results are expressed as 10⁻³ international enzyme units per mg of cells.

was added, and after 5 min the absorbancy at 340 nm was read again. Experimental values were corrected for NADH disappearance in blank incubation mixtures without O-succinylhomoserine. For a given sample of toluenized cells, net NADH oxidation is proportional to the quantity of cells. Cells of a metB⁻ mutant (cystathionine synthetaseless) gave no activity in the assay. In separate experiments, toluenized cells and sonic extracts gave the same cystathionine synthetase activities. Cystathionase was assayed by a modification of the procedure of Flavin (6) by measurement of the rate of cystathionine-dependent reduction of Ellman's reagent [5,5′ dithio-bis-(2-nitrobenzoic acid); (5)] at room temperature at 410 nm with a Gilford model 2000 recording spectrophotometer. Assay mixtures contained: 1 µmole of L-cystathionine; 0.125 µmole of $MgSO_4$, 160 µmoles of potassium phosphate, pH 7.4; 1 µmole of Ellman's reagent, and 0.1 ml
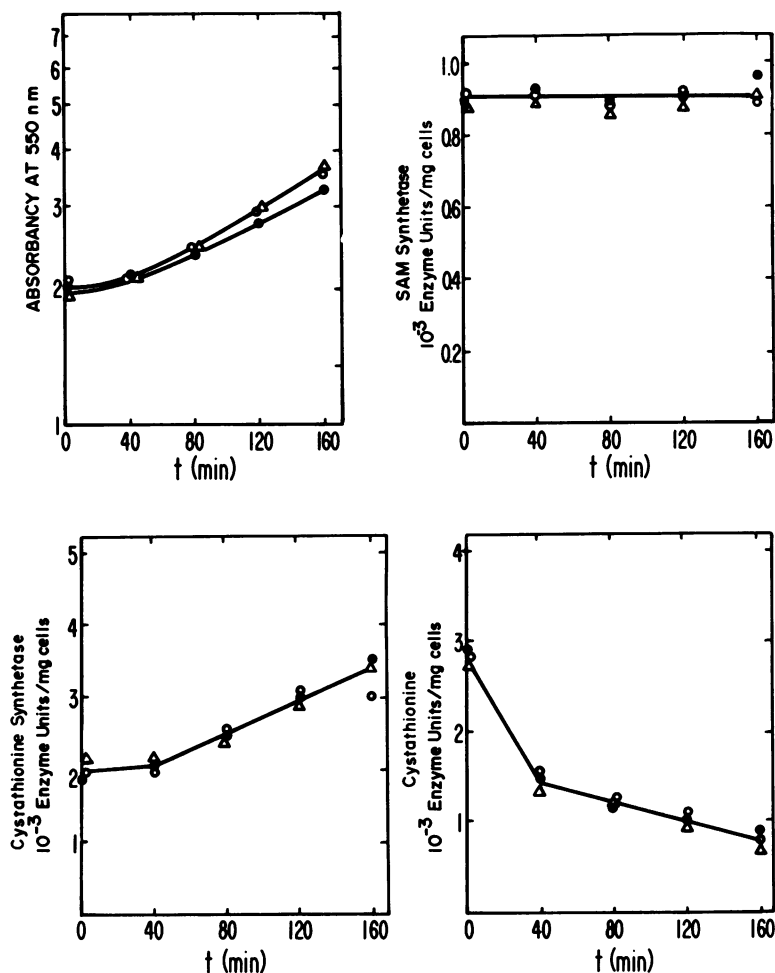
FIG. 5. *Effect of methionine on enzyme synthesis in strain D8. D8 cells grown to stationary phase on LB broth were incubated in 5 × 10⁻³ M L-threonine-supplemented minimal dextrose medium with the addition of (●) no methionine, (○) 10⁻³ M L-methionine, or (△) 5 × 10⁻³ M L-methionine.*

of toluenized cells in a total volume of 1 ml. Each experimental value was corrected for a blank incubation without cystathionine. The rate of cystathionine cleavage is directly proportional to the amount of a given toluenized cell preparation added to the incubation mixture. Cells of a metC⁻ mutant (cystathionase-less) had negligible activity. When stationary-phase cells are used, the toluenized cell assay gives slightly lower cystathionase activities (70 to 90%) than is obtained with sonic extracts. When using the toluenized cell assay, we noted that cystathionase activity dropped on incubation of constitutive cells in minimal medium. When these assays were repeated with sonic extracts, no drop in activity was seen, indicating that some change which influenced the toluenized cell assay (possibly accumulation of an inhibitor which does not diffuse out of toluenized cells) had occurred, rather than a loss in enzyme activity. Thus increases in cystathionase may be masked, and the toluenized cell assay

can be used only for qualitative comparison of cells with relatively large differences in activity.

Uptake measurement. Log-phase cells were harvested by centrifugation and suspended in sufficient minimal medium (0.5% dextrose) to give an absorbance of 10 to 12 at 550 nm. Samples (0.9 ml) of cells were warmed at 37 C for 5 min, and 0.1 ml of a 10⁻⁴ M solution of labeled compound was added. At indicated times, 0.1-ml samples were pipetted into tubes containing 5 ml of minimal medium at room temperature, the contents were mixed with a Vortex mixer and were filtered through a 25-mm membrane filter (0.45 μm pore size; Millipore Corp.) using a sampling manifold (Millipore model 3025). The tube and filter were rinsed with an additional 2 ml of medium. The first filtration was finished about 15 sec after taking the sample, and the wash took another 10 to 15 sec. Controls without cells and with boiled cells were done to measure nonspecific binding of label. Duplicate sam-
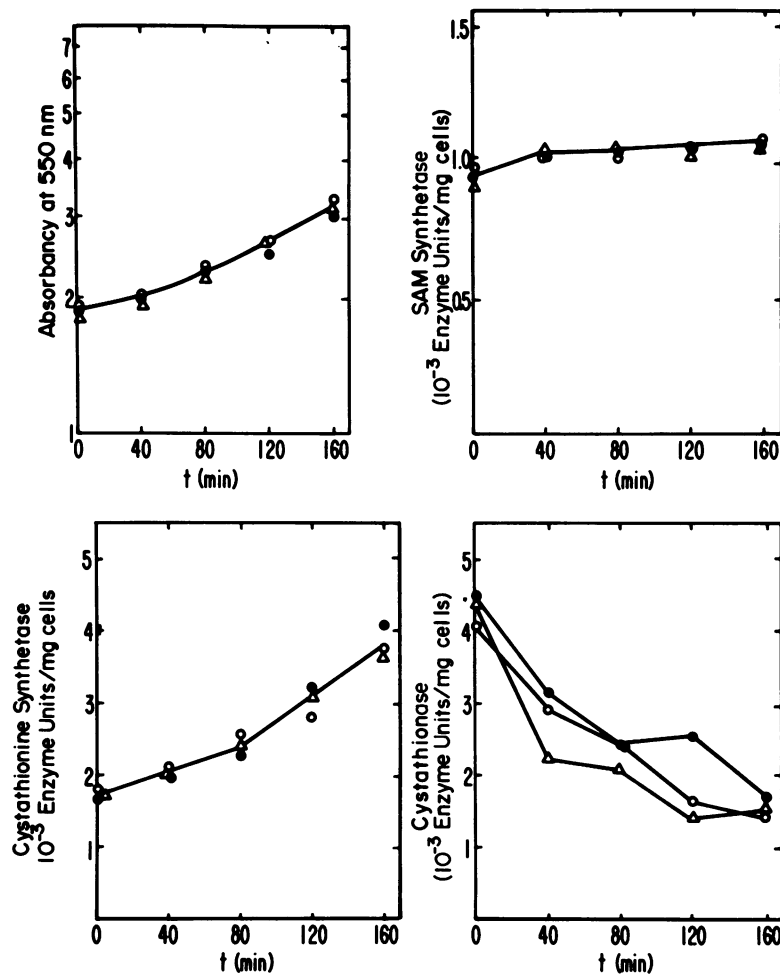
FIG. 6. *Effect of methionine on enzyme synthesis in strain E31. E31 cells grown to stationary phase in LB broth were incubated in 5 × 10⁻³ M L-threonine-supplemented minimal dextrose medium with the addition of (●) no methionine, (○) 10⁻³ M L-methionine, or (△) 5 × 10⁻³ M L-methionine.*

ples (0.02 ml) of each suspension were pipetted onto discs (Millipore) to measure the radioactivity in the suspension. The discs were placed in counting vials and dried overnight in a vacuum desiccator. Five milliliters of scintillation fluid [0.4% 2,5-diphenyl-oxazole; 0.01% 1,4-bis-2-(5-phenyloxazolyl) benzene in toluene] was added to each vial, and the samples were counted in a Packard TriCarb model 3375 liquid scintillation spectrometer.

RESULTS

In our initial experiments stationary-phase cells which had been grown on minimal medium containing dextrose had high levels of SAM synthetase. When such cells were diluted into fresh minimal medium, the specific activity of the SAM synthetase dropped during the early stages

of growth and then rose as the cells entered late log phase. In medium containing methionine, the specific activity dropped to lower values and the subsequent rise in specific activity was delayed. Thus it appeared that addition of methionine to the growth medium caused repression rather than induction of SAM synthetase.

Since derepression can be studied best in cells with low initial specific activities, several media were evaluated for growth of stationary-phase cells with acceptably low enzyme activities. The level of SAM synthetase in stationary-phase cells is a function of many parameters, including the nature of the carbon source and the final pH of the medium as well as amino acid supplementation. No attempt has been made to systematically evaluate these parameters, but cells grown
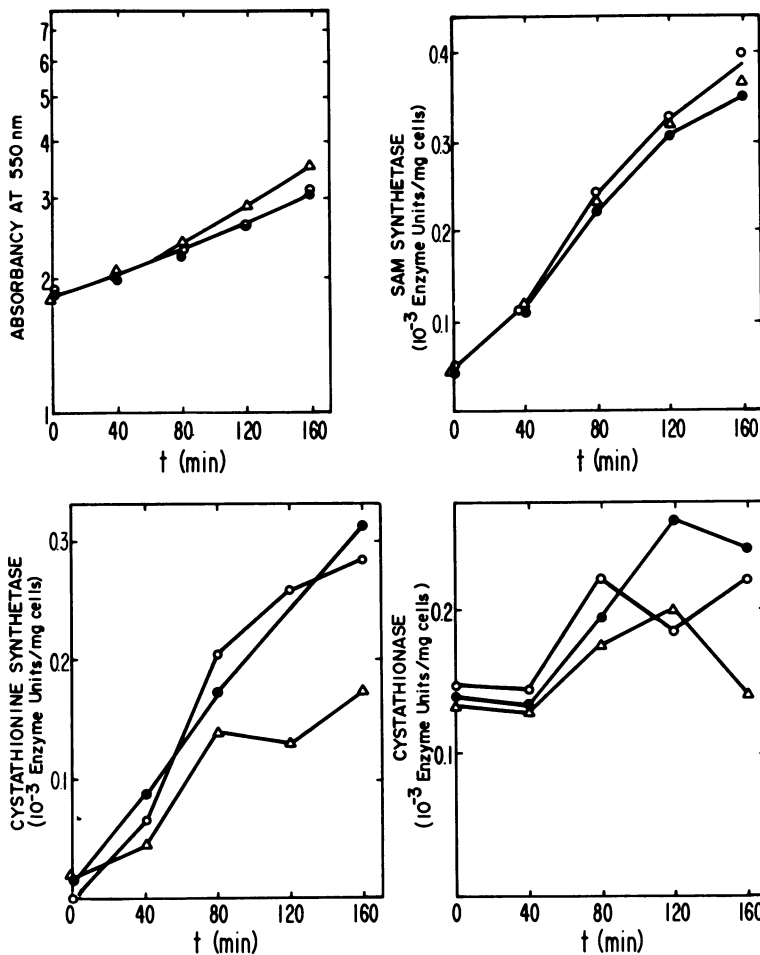
FIG. 7. *Effect of ethionine on derepression. Wild-type cells grown to stationary phase on LB broth were incubated in threonine (5×10^{-3} M) containing minimal dextrose medium with (●) no addition, (○) 2×10^{-4} M L-ethionine, or (△) 10^{-3} M L-ethionine.*

in complete media generally have lower activity than those grown in defined media. The lowest activity was obtained in cells grown in LB broth, so these cells were routinely used in derepression experiments. When it is desired to use cells grown on defined media, somewhat higher but acceptably low activities are obtained with cells grown in minimal medium containing 0.5% sodium lactate in place of dextrose as the carbon source and supplemented with mM L-methionine.

Cells with low specific activities obtained by growth to stationary phase on LB broth were harvested, suspended in minimal medium, and incubated alone or with methionine supplementation. Figure 1 shows the growth and enzyme specific activities of these cells. The growth behavior shows the lag that is charac-

teristic for cells grown in rich medium and transferred to an unsupplemented medium. In the absence of methionine the specific activities of all three enzymes increase with time, but, as has been reported for the enzymes of the methionine biosynthetic pathway, the increases are not coordinate (6). Cystathionine synthetase and SAM synthetase both increase about 10- to 12-fold whereas cystathionase shows only a small increase in activity. Both levels of methionine supplementation prevent any increase in enzyme activities for the first 4 hr of incubation. After this time, the cells in the medium supplemented with 2×10^{-4} M L-methionine are released from repression (probably because of depletion of the exogeneous supply of methionine) with cystathionine synthetase showing the most rapid increase in specific activity and cystathionase showing the
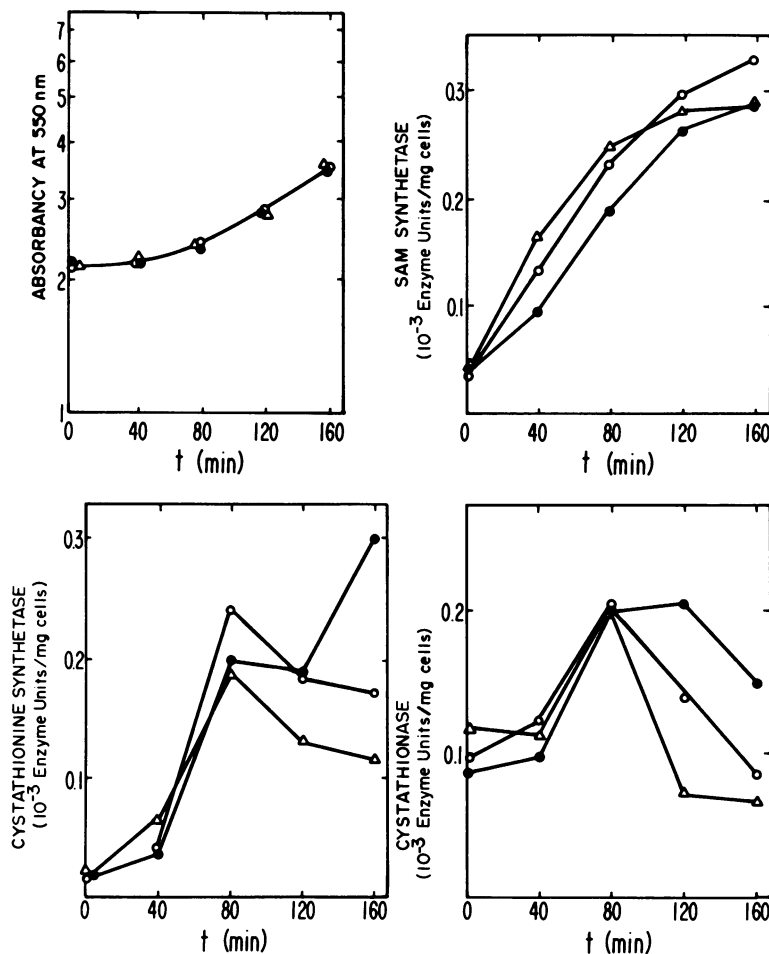
Fig. 8. *Effect of norleucine on derepression. LB broth-grown wild-type cells were incubated in minimal dextrose medium supplemented with 5 × 10⁻³ м L-threonine and* (●) *no further addition,* (○) *2 × 10⁻⁴ м L-norleucine, or* (△) *10⁻³ м L-norleucine.*

slowest increase. Little if any derepression is seen during the 8-hr incubation period in cells grown on medium supplemented with mM L-methionine.

Although these experimental conditions allow demonstration of substantial increases in enzyme activity, which are prevented by addition of methionine to the growth medium, the long lag period before derepression is inconvenient. Attempts to reduce the intracellular methionine pool and thus speed up derepression were made by addition of threonine and lysine to the growth medium, since these amino acids inhibit the activities of the aspartokinase I, homoserine dehydrogenase I complex and aspartokinase III in *E. coli* K-12 (17, 18, 29). The early stages of derepression of cells pregrown in LB broth are not affected by addition of 5 × 10⁻³ м L-lysine

to the incubation medium. During the later stages lysine does cause an increase in enzyme activities above those of the control, but this effect has not been further investigated. On the other hand (Fig. 2), 5 × 10⁻³ м L-threonine has a marked effect on growth and derepression of LB broth-grown cells. Unlike strain Hfr H, which is inhibited by threonine (17), the growth of the strain used in these experiments is stimulated by threonine. The time course of derepression of the enzymes is also accelerated by addition of threonine to the growth medium, with the onset of enzyme synthesis preceding rapid growth in media either with or without threonine. Because of the marked growth stimulation, apparently, in this strain, the ability to synthesize threonine limits protein synthesis after transfer from LB broth to minimal medium. Threonine
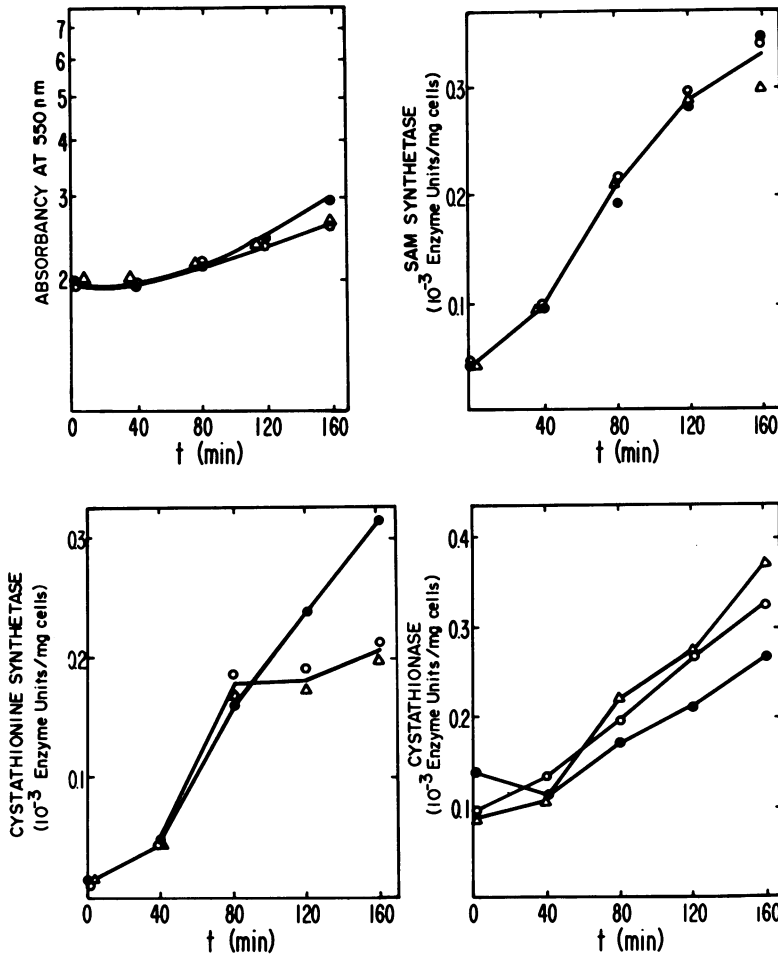
FIG. 9. Effect of α-methylmethionine on derepression. Wild-type cells grown on LB broth were incubated in 5 × 10⁻³ M L-threonine supplemented minimal dextrose medium containing, (●) no α-methylmethionine, (○) 2 × 10⁻⁵ M DL-α-methylmethionine, or (△) 6 × 10⁻⁵ M DL-α-methylmethionine.

has a much smaller effect on cells, pregrown in in minimal-sodium lactate-methionine medium, which do not show a lag in growth or derepression. Regardless of the basis of the threonine effect, incubation of LB broth-grown cells in medium containing 5 × 10⁻³ M L-threonine provides a convenient system for evaluation of the repressive effects of methionine and its analogues. The more rapid derepression accompanied by relatively little growth allows demonstration of the repressive effects of much lower concentrations of methionine. As shown in Fig. 3, 2 × 10⁻⁵ M L-methionine clearly causes repression for 80 to 120 min, after which enzyme synthesis begins, probably reflecting depletion of methionine. Figure 4 shows that chloramphenicol (40 μg/ml) stops growth and prevents the increases in enzyme activities, which indicates that

protein synthesis is required for the increases in enzyme activity, and the phenomenon probably represents derepression.

Since the development of enzyme activities are not coordinate, there was no indication that a common control system is involved in the regulation of their synthesis until several constitutive mutants with elevated activities of all three enzymes were isolated. Table 1 shows the enzyme activities of stationary-phase, LB broth-grown cells of the wild-type and constitutive strains. The activities of all three enzymes are markedly increased in the mutants D8 and E31 when incubated in threonine containing minimal medium alone or with the addition of methionine (Fig. 5 and 6). Although there is an increase in the specific activity of cystathionine synthetase and an apparent decrease in that of cystathionase during
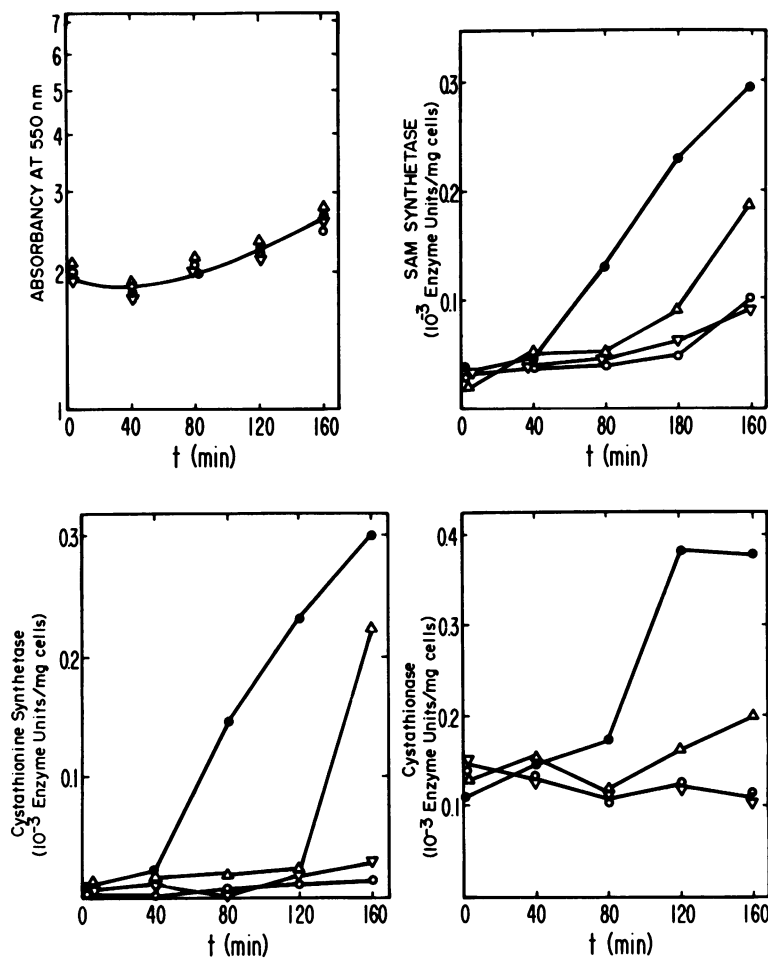
FIG. 10. *Comparison of the repressive effects of methionine and selenomethionine. Wild-type cells grown to stationary phase in LB broth were incubated in threonine (5×10^{-3} M) containing minimal dextrose medium with* (●) *no additions,* (○) 4×10^{-5} M *DL-selenomethionine,* (△) 4×10^{-5} DL-*methionine, or* (▽) 10^{-4} M DL-*methionine.*

the incubation, these changes are not influenced by methionine at a concentration (5×10^{-3} M) 250 times greater than that (2×10^{-5} M) which causes significant repression in wild-type cells.

Addition of chloramphenicol (40 µg/ml) to the growth medium prevents the increase in specific activity of cystathionine synthetase but does not prevent the apparent drop in activity of cystathionase. This decrease represents some peculiarity in the toluenized cell assay procedure rather than a loss of enzyme.

Figures 7 to 9 show the effects of ethionine, norleucine, and α-methylmethionine on growth and derepression of wild type cells. While ethionine and norleucine can be used at relatively high concentrations, α-methylmethionine concentra-

tions higher than those shown inhibit growth, and the results are complicated by its inhibition of methionyl transfer ribonucleic acid (tRNA) synthetase (26). None of these compounds has an appreciable effect on the enzyme activities compared to that of methionine. Ethionine at 10^{-3} M exhibits slight repression of cystathionine synthetase, and norleucine (both at 2×10^{-4} and 10^{-3} M) slightly stimulates SAM synthetase formation. These differences are small and may not be significant. Selenomethionine, on the other hand, is slightly more effective at preventing enzyme synthesis than an equal concentration of methionine. As shown in Fig. 10, 4×10^{-5} M DL-selenomethionine represses as well as 10^{-4} M DL-methionine, and both show

more prolonged repression than 4×10^{-5} M DL-methionine.

Because of indications that the corepressor for control of the methionine biosynthetic enzymes might be SAM or one of its metabolites, the reactivity of the analogues with SAM synthetase was measured to see whether there was any correlation between the repressive activity of a compound and its ability to be converted to an adenosyl derivative. As shown in Table 2, the activity of *E. coli* SAM synthetase is approximately the same in the presence of 0.01 and 0.02 M L-methionine, indicating that the enzyme is saturated in this concentration range. A small amount of SAM is synthesized in the reaction mixtures containing D-methionine, but, since the optical purity of the D-methionine was not tested, the observed activity may be due to L-methionine contamination of the material used. Regardless of its reactivity with the enzyme, D-methionine does not appreciably inhibit the reaction with L-methionine. Ethionine is a relatively poor substrate for *E. coli* SAM synthetase, whereas norleucine and α-methylmethionine do not appear to be substrates at all. In contrast to the other analogues, selenomethionine reacts with the enzyme more than twice as rapidly as methionine. Thus the analogues which are ineffective at repression are not good substrates for SAM synthetase, whereas one analogue, selenomethionine, which reacts with the enzyme better than methionine, is also slightly more effective at repression.

With this correlation in mind, the ability of SAM to repress enzyme formation was tested. As shown in Fig. 11, addition of SAM to the incubation medium during derepression of cells pregrown in LB broth has no effect on the development of enzyme activity. Since equivalent concentrations of methionine caused significant repression under these conditions, these results tended to rule out SAM as an effector. Before such a conclusion could be drawn, however, it was necessary to measure the uptake of SAM by the cells. Figure 12 shows the results of one such uptake experiment. Radioactivity corresponding to approximately 5 nmoles of SAM (adenine-*8*-^{14}*C*) was concentrated per gram of cells when the rapid stage of uptake was completed. In a control experiment with the same cell suspension, the methionine transport system was shown to be functioning. It is questionable whether any SAM was taken up, since the amount of label bound to the cells (1.5%) was less than the radioactive impurities in the SAM preparation (2.5%). Preliminary estimates of the SAM pool

TABLE 2. *Reaction of methionine and analogues with SAM synthetase*[a]

Substrate	Activity[b]
0.01 M L-Methionine	31.5
0.02 M L-Methionine	31.9
0.02 M D-Methionine	2.1
0.02 M DL-Methionine	30.5
0.04 M DL-Methionine	31.7
0.02 M L-Ethionine	7.2
0.02 M DL-α-Methylmethionine	0
0.02 M L-Norleucine	0
0.02 M DL-Selenomethionine	65.6

[a] The incubation procedure described in the text was followed except that the ATP-*8*-^{14}*C* had twice the normal specific activity (0.05 μCi/μmole) and each incubation mixture contained 6 mg of toluenized E31 cells grown to stationary phase in minimal dextrose medium.

[b] Results expressed as nanomoles per 30 min per milligram of cells.

in wild-type cells during log phase in minimal medium are in the range of 30 to 50 nmoles/g. Thus, even if SAM were taken up, the change in the pool size would be too small to cause measurable repression, and no conclusions about the role of SAM in enzyme regulation can be drawn from these experiments.

DISCUSSION

SAM synthetase was reported to be inducible by methionine in yeast (20), but its control has not been previously studied in *E. coli*. The results presented here clearly indicate that the enzyme is repressed by addition of methionine to the growth medium. The mutants used in these experiments cotransduce with *metB* markers with high efficiency suggesting that they are in the *metJ* locus, which was described as a methionine regulatory gene in *S. typhimurium* (13). Since these mutants are constitutive for cystathionine synthetase and cystathionase (and by inference for the other enzymes of methionine biosynthesis) as well as being constitutive for SAM synthetase, it is reasonable to conclude that SAM synthetase is regulated by the same system that controls the enzymes of methionine biosynthesis.

The relative abilities of the methionine analogues to cause repression of enzyme synthesis may reflect directly their reactivities with the control system or indirectly their abilities to be converted to compounds which in turn react with the control system. MetG mutants of *S. typhimurium*, which have been shown to have an
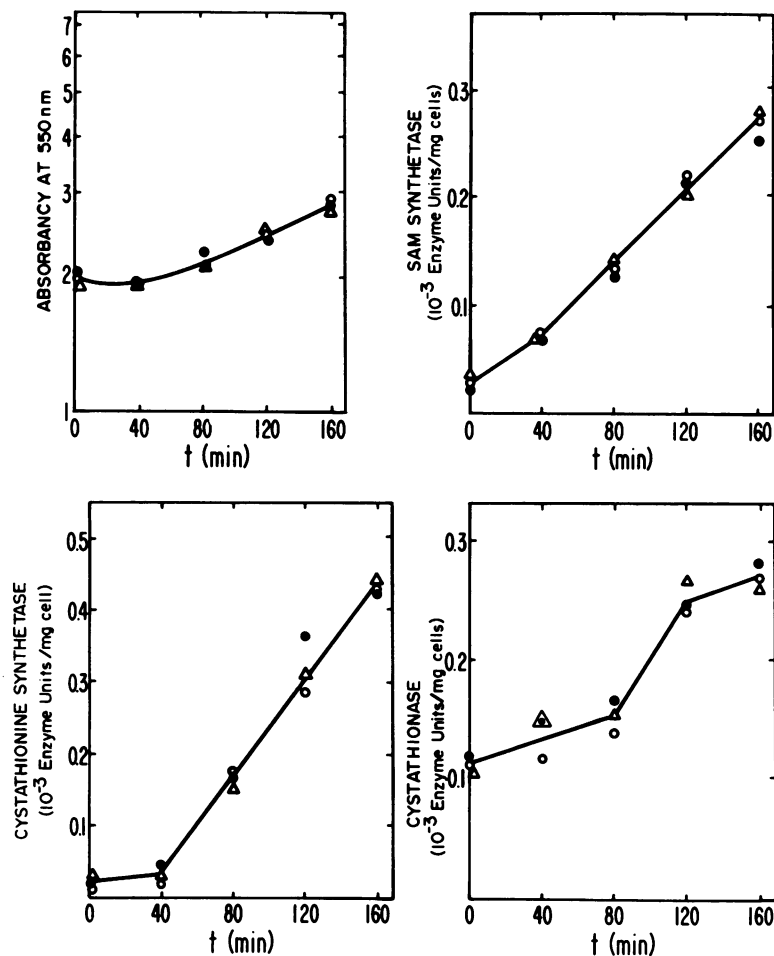
FIG. 11. *Effect of SAM on enzyme synthesis.* LB broth-grown wild-type cells were incubated in minima dextrose medium supplemented with 5×10^{-3} M L-threonine and (●) no further additions, (○) 2×10^{-5} M SAM or (△) 6×10^{-5} M SAM.
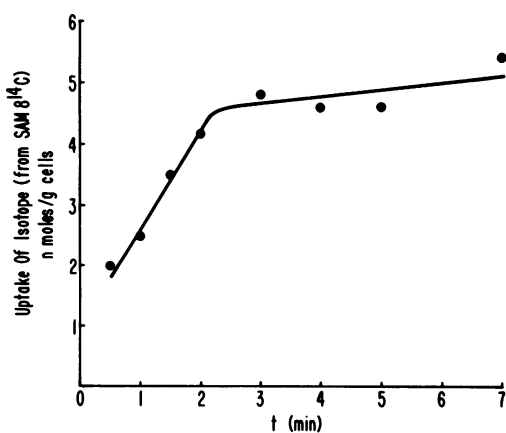

FIG. 12. *Uptake of SAM.* Wild-type cells were grown in minimal dextrose medium at 37 C to an absorbancy (550 nm) of 1.8. The cells were harvested and suspended in fresh minimal dextrose medium to give a suspension with an absorbancy (550 nm) of 12.6 (~19 mg/ml). The suspension (0.9 ml) was incubated at 37 C for 5 min and 0.1 ml of 10^{-4} M SAM (8-^{14}C) was added at zero time. Samples were taken.

altered methionyl (tRNA) synthetase (10), are not derepressed for the methionine biosynthetic enzymes, suggesting that methionyl tRNA is not involved in repression. On the other hand, several groups of workers (1, 12, 14, 21) have reported the involvement of SAM in regulation of methionine synthesis, mostly at the level of feedback inhibition. Kerr and Flavin (12) recently showed that an ethionine-resistant mutant of *Neurospora crassa*, originally isolated by Metzenberg (15), has low SAM synthetase and overproduces methionine. They propose that in this strain, the methionine overproduction results from release of feedback inhibition rather than derepression. In contrast, we have reported (9) that low SAM synthetase mutants of *E. coli* K-12 have elevated levels of cystathionine synthetase and cystathionase (and presumably of the other methionine biosynthetic enzymes). The behavior of these strains as well as the results with analogues of methionine are consistent with the hypothesis that the effector in repression of the methionine biosynthetic enzymes in *E. coli*

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is SAM or one of its metabolites. Unfortunately, the impermeability of E. coli K-12 to SAM prevents a direct test of this hypothesis.

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