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Regulation of Methionyl-Transfer Ribonucleic Acid Synthetase Formation in *Escherichia coli* and *Salmonella typhimurium*

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The control of methionyl-transfer ribonucleic acid (tRNA) synthetase (L-methionine: soluble RNA ligase [adenosine monophosphate]) was studied in methionyl-tRNA synthetase mutants of *Escherichia coli* and *Salmonella typhimurium*. The results of activity determinations with crude extracts indicate that this enzyme of the *E. coli* mutant strain possessed a reduced affinity for methionine-tRNA, whereas this enzyme of the *S. typhimurium* mutant exhibited a decreased affinity for L-methionine. The differential rate of methionyl-tRNA synthetase formation in these two mutants was several-fold greater than that of the respective parental strains. On the other hand, the level of in vivo aminoacylation of methionine-tRNA was only about one-third that of the parent strains. These results suggest that aminoacylation of methionine-tRNA is a necessary step in repression control of methionyl-tRNA synthetase of both *E. coli* and *S. typhimurium* strains.

It has been suggested from recent studies in our laboratory that the control of aminoacyl-transfer ribonucleic acid (tRNA) synthetase formation involves a repression-like mechanism (1, 5, 7, 11). Consistent with these findings, methionyl-tRNA synthetase formation was shown to be regulated by a process which was affected by manipulating the methionine supply to the cells (1). The results of similar studies with the amino acids arginine, histidine, valine, leucine, isoleucine, proline, and threonine suggested an involvement of these amino acids in regulating the synthesis of the cognate aminoacyl-tRNA synthetases (5, 6, 7, 11).

In addition, data obtained from the study of histidine regulatory mutants of *Salmonella typhimurium* suggest that histidyl-tRNA, rather than histidine, is the physiologically significant unit for repression control of synthesis of this synthetase (7).

In view of these findings (7), we designed studies to ascertain the possible involvement of methionine-tRNA ($tRNA^{met}$) in the control of its cognate synthetase.

MATERIALS AND METHODS

Organisms. Two strains of *Escherichia coli* and two strains of *S. typhimurium* were used in these studies. NP2 is a K-12 wild-type strain of the Neidhardt-Purdue stock. NP207 is an L-ethionine-resistant mutant derived from strain NP2 by ethylmethane sulfonate mutagenesis (8). The *S. typhimurium* strains were SPI (*ara-9 metE338 ilvC401 strA149 his3179*) and SPIII (*ara-9 metG319*), both of which were originally obtained from P. E. Hartman.

Media and methods of cultivation. The minimal medium was the basal salts solutions of Fraenkel and Neidhardt (3) supplemented with 0.4% glucose and 0.2% ammonium sulfate as carbon and nitrogen sources. Deuterium oxide (D_2O) minimal medium was prepared in the same manner, except that water was replaced by 100% D_2O. For unrestricted growth of these organisms, 100 μg of all required amino acids (L-isomer form) per ml were used. For restricted growth conditions, the cells were grown in minimal glucose medium. All other required amino acids were supplied at a concentration of 100 μg/ml. Unless otherwise noted, the cells were grown aerobically on a rotary action shaker at 37 C. In each case, the cells were grown overnight in unrestricted medium and transferred to a medium of the same composition prior to the initiation of each experiment. Growth was measured by an increase in optical density at 420 nm with 1-cm light path in a Zeiss PMQ II or Hitachi-Perkin-Elmer, model 101, spectrophotometer.

Isolation of ethionine-resistant mutant. The general isolation procedure was as follows. Mutagenesis was carried out by treating 10-ml samples of cells grown overnight in minimal medium containing methionine (100 μg/ml) with two drops of ethyl methane sulfonate for 2 h at 37 C, after which 50 ml of

fresh medium was added, and the culture allowed to grow (8). The cells were then collected by centrifugation, washed twice with minimal medium, resuspended in 1/10 the original volume by using sterile minimal medium, and plated on minimal medium plates containing L-ethionine (200 μg/ml). The plates were incubated at 37 C for 48 h and examined for appearance of ethionine-resistant colonies. Such colonies were placed in liquid culture and tested for growth resistance to L-ethionine and checked for parental characters.

Preparation of cell extracts. Cells were subjected to sonic treatment with a Biosonik III Sonifier as described by Chrispeels et al. (2). The protein content was determined colorimetrically by the methods of Lowry et al. (9).

Preparation and oxidation of tRNA. Cells were grown exponentially in minimal glucose medium, and 80-ml samples were taken at each one-half mass doublings for two generations of growth. Samples were immediately added to tubes containing 20 ml of 100% trichloracetic acid. After centrifugation at 4 C, the resulting pellet was suspended in 0.05 M sodium acetate buffer (pH 5.5) containing 0.06 M KCL and 0.01 M $MgCl_2$. The cells were subjected to sonic treatment, phenol extraction, and ethanol precipitation as described by Williams and Freundlich (10). The tRNA preparations were subjected to periodate oxidation and were deacylated of amino acids by incubation for 1 h in 0.1 M tris(hydromethyl)-aminomethane-hydrochloride (pH 8.9) at 37 C as described earlier (10). The RNA content of the samples was determined by measuring absorbance at 260 nm, assuming 24 optical density units are equal to 1 mg of RNA per ml. The tRNA samples were used in the standard attachment assay system as described elsewhere (2).

Enzyme measurement. The activity of methionyl-tRNA synthetase was determined by the ^{14}C-labeled amino acid attachment assay system as described by Chrispeels et al. (2). Specific activity was expressed as units per milligram of protein, with one unit being defined as 1 μmole of amino acid attached to tRNA per h. For all differential plots of the results, the rate of synthesis was determined from the slope of the lines in each plot.

Measurement of the de novo rate of enzyme synthesis. All procedures were as described by Williams and Neidhardt (11).

Chemicals. All chemicals were of the highest grade available. Uniformly labeled L-[^{14}C]- and DL-[^{14}C]-amino acids were obtained from Schwarz Bioresearch Inc. (Orangeburg, N.Y.). D_2O (99.8%) was purchased from Volk Radiochemical Co. (Burbank, Calif.). L-Ethionine was purchased from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

Methionyl-tRNA synthetase formation in an ethionine-resistant mutant of E. coli. Cells were initially grown exponentially in minimal medium supplemented with methionine (100 μg/ml), harvested by centrifugation, washed with minimal medium, and suspended in minimal-glucose medium. During growth in minimal medium, the growth rate of the ethionine-resistant mutant, NP207, was 60 to 70% of the growth rate of the wild-type strain (Fig. 1). The first question asked was what is the rate of methionyl-tRNA synthetase formation in this mutant as compared to the parent strain. The results of experiments designed to address this question are presented in Fig. 2A and 2B. In Fig. 2A, it can be seen that the differential rate of methionyl-tRNA synthetase formation was several-fold greater than that of the wild-type strain during growth in minimal medium. As expected, arginyl-tRNA synthetase formation occurred at essentially the same rate in both strains. In addition, we determined the rate of methionyl-rRNA synthetase formation by use of density labeling involving deuterium oxide to water media shifts. The results shown in Fig. 2B indicate that the de novo rate of formation of this enzyme in this ethionine-resistant mutant was increased several-fold as compared to that of the wild-type strain. Importantly, this isolate of strain NP2 possessed all parental characters except growth resistance to the methionine analogue, ethionine. What then is the basis for the elevated rate of methionyl-tRNA synthetase formation?

The obvious possibilities were considered

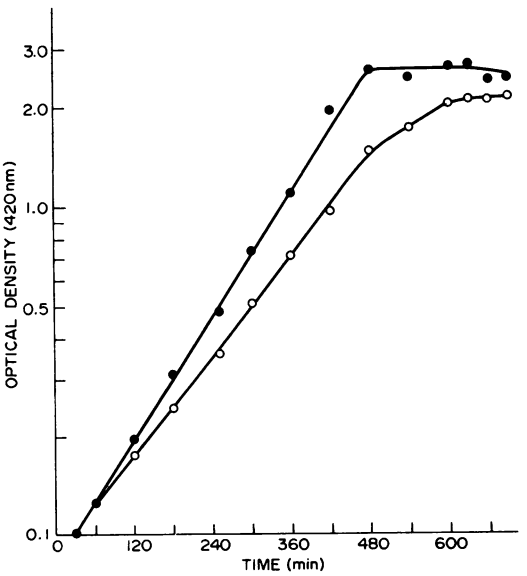

FIG. 1. *The growth of E. coli strains NP2 and NP207. The cells were grown overnight in unrestricted medium and transferred to minimal glucose medium. Growth was determined from the increase in optical density at 420 nm for the wild type (NP2, ●) and the mutant (NP207, ○) strains.*

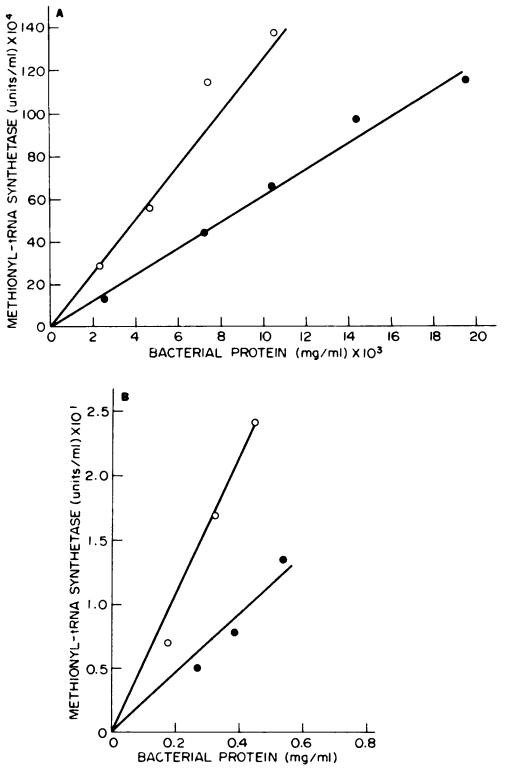

FIG. 2. *A, The rate of formation of methionyl-tRNA synthetase during steady-state growth in minimal medium. The cells were grown exponentially in minimal medium, and sampling was as described in Materials and Methods. The results are expressed as enzyme units per milliliter as a function of total protein per milliliter of culture for NP2 (●) and NP207 (○). B, Differential rate of formation of methionyl-tRNA synthetase as determined by density labeling. Experimental conditions are the same as those described for A. These results were obtained from the rate of appearance of light enzyme units in the cultures as described in Materials and Methods for strains NP2 (●) and NP207 (○).*

(i.e., altered L-methionine permease system, a methionine regulatory mutant, altered methionyl-tRNA synthetase or $tRNA^{met}$). In an effort to delineate between these alternatives, activity determinations were made for the affinity of methionyl-tRNA synthetase of both strains for the substrates, adenosine triphosphate (ATP), L-methionine, and $tRNA^{met}$. The K_m values for ATP and L-methionine were found to be essentially the same for the synthetase of both strains. However, the mutant strain possessed a methionyl-tRNA synthetase with reduced affinity for methionine tRNA (Fig. 3). Consistent with this observation, the in vivo level of aminoacylation of $tRNA^{met}$ of this mutant strain during growth in minimal medium was only about 40% of the value observed for the wild-type strain (Table 1). Although these determinations (Fig. 3) were performed with crude extracts, they are, at least, suggestive of the fact that a methionyl-tRNA synthetase with an altered affinity for $tRNA^{met}$ was the basis for the two phenotypic characters (i.e., ethionine resistance and derepressed rate of methionyl-tRNA synthetase formation).

To further examine the response of the control signal for methionyl-tRNA synthetase formation, we grew the cells under different nutritional conditions. Both strains were grown overnight in minimal medium, washed, and transferred to the same medium, and growth was allowed to proceed for 75 min. There was an increased methionyl-tRNA synthetase activity in both strains during the transition from stationery to exponential cells (Fig. 4). However, in both instances, the mutant strain possessed a higher level of this enzyme than the wild-type strain (Fig. 4). Upon a transfer of these cells to nutrient broth medium, there was a reduction in the level of activity of this synthetase in both strains; however, even under these enriched growth conditions, the ethionine-resistant mutant maintained an elevated level of methionyl-tRNA synthetase activity as compared to the normal strain (Fig. 4). These results (Fig. 4) suggest that the increased rate of methionyl-tRNA synthetase formation in this mutant was

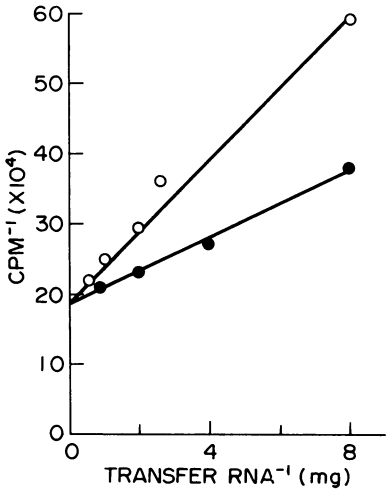

FIG. 3. *K_m determinations of methionyl-tRNA synthetase for methionine tRNA for the wild type (NP2) and the mutant (NP207) strains. The determination was made by using the standard attachment assay system. Increasing amounts of tRNA was added each reaction tube. ^{14}C-methionine, ATP, and extracts were in excess amounts. The results are shown for the wild type (●) and the mutant (○).*

TABLE 1. *In vivo level of charging of methionine t-RNA in strains NP2 and NP207*[a]

Strain	Mass increase(%)	Sp ac[b]	Relative charging of $tRNA^{met}$ (%)
NP2	0	0.34	89
	50	0.30	92
	100	0.36	98
NP207	0	0.47	62
	50	0.65	48
	100	0.65	37

[a] Sampling, extraction, and precipitation of tRNA were as described in Materials and Methods. The acceptance activity was determined for the control and periodate-treated samples by using the standard attachment assay system.

[b] Specific activity for methionyl-tRNA synthetase is expressed as units of enzyme per milligram of protein.

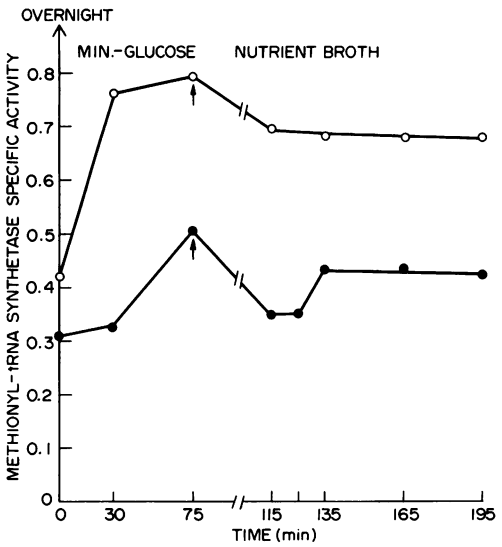

FIG. 4. *Activity of methionyl-tRNA synthetase during growth in different media. The cells were grown exponentially in minimal medium. At the time indicated by the arrow, the cells were transferred to nutrient broth medium. Samples were collected, and the activity was determined for the wild type (NP2, ●) and mutant (NP207, ○) strains.*

not due to a restricted supply of L-methionine or any other components which can be supplied by growth medium supplementation. Thus, these data (Fig. 2-4) suggest that the derepressed rate of synthesis of methionyl-tRNA synthetase was a consequence of an alteration in the synthetase leading to a reduced affinity for $tRNA^{met}$, which lowered the in vivo level of charged $tRNA^{met}$, and caused a derepression of synthesis of this synthetase.

Synthesis of methionyl-tRNA synthetase in a metG mutant of S. tryphimurium. In view of the results obtained with the ethionine-resistant mutant, we examined methionyl-tRNA synthetase formation in a methionyl-tRNA synthetase (*metG*) mutant of *S. typhimurium*. During steady state growth in minimal medium, the *metG* mutant grew at a rate equal to 20% of that of the normal strain (Fig. 5). Upon the addition of excess methionine to the growth medium, the mutant strain resumed a growth rate comparable to that of the normal strain (Fig. 5). An explanation for the growth response of this *metG* mutant was provided by determination of the affinity of methionyl-tRNA synthetase of this mutant for ATP, L-methionine, and $tRNA^{met}$. The K_m values for ATP and $tRNA^{met}$ were the same as those observed for the enzyme of the normal strain, SPI. However, there was a significant reduction in the affinity of the mutant enzyme for L-methionine (Fig. 6).

For determination of the rate of methionyl-tRNA synthetase formation in these two strains, the normal strain, a methionine auxotroph, was grown in minimal medium supplemented with L-methionine (100 μg/ml); strain 1365, the *metG* mutant, was grown in minimal medium. The differential rate of synthesis of this synthetase in the *metG* mutant was three times greater than that of the $metG^+$ strain (Fig. 7). The addition of excess methionine to a culture of the *metG* mutant resulted in a comparable rate of methionyl-tRNA synthetase formation as observed for the $metG^+$. The methionine auxotroph grew at the unrestricted growth rate, whereas growth of mutant strain SP III was limited (Fig. 5). This limited growth of this *metG* mutant was due to the concentration (less than K_m) of L-methionine for the methionyl-tRNA synthetase. In fact, the same growth and activity responses were observed with a methionine prototroph and strain SPIII (*metG* mutant) grown in minimal medium. Further evidence that the rate of methionyl-tRNA synthetase formation in this mutant can be varied by the supply of L-methionine to the cells is provided in Fig. 8. In this experiment, the *metG* mutant strain was grown in minimal medium containing L-methionine (100 μg/ml) and was washed twice with minimal medium. The culture was divided into two flasks, one containing minimal medium alone and the other minimal medium supplemented with L-methionine (100 μg/ml). Upon the transfer to minimal medium, there was an immediate increase in the specific activity of this synthetase from 0.28 to 0.80 units per mg of protein (Fig. 8). Significantly, restoration of excess L-methionine to this culture cause a repression

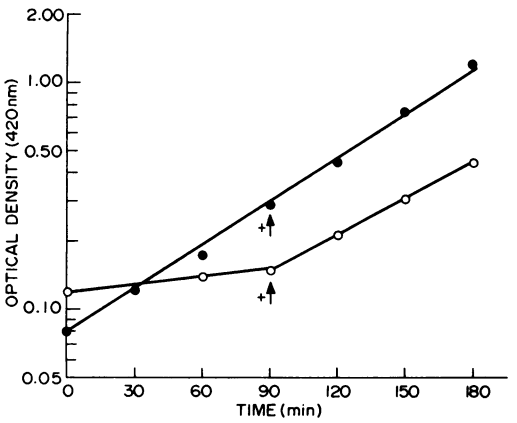

FIG. 5. *Growth of Salmonella typhimurium strains SB109 and SB1365 in minimal medium. SB1365* (O) *was grown in minimal medium; and at the time indicated by the arrow 100 μg of methionine per ml was added to the culture. Strain SB109* (●) *was grown in minimal medium containing* L-*methionine (100 μg/ml). Growth was determined as described in Fig. 1.*

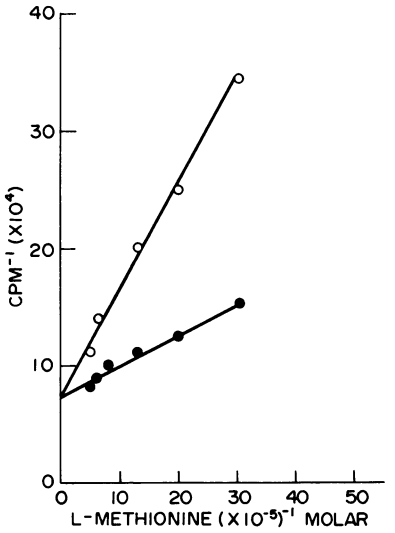

FIG. 6. *Determination of K_m value for* L-*methionine of methionyl-tRNA synthetase in crude extracts of strains SB109 and SB1365. The determinations were performed as described in Fig. 3, by using increasing concentrations of* ^{14}C-*methionine for wild-type methionyl-tRNA synthetase* (●) *and the mutant synthetase* (O).

(decrease in specific activity) to nearly the original level characteristic of growth in excess L-methionine. The maintenance of a fairly constant specific activity during continued growth in methionine-unrestricted medium indicated that the considerable increase (derepression) in methionyl-tRNA synthetase activity was specifically related to the methionine supply to these cells (Fig. 8). Furthermore, examination of the in vivo aminoacylation of $tRNA^{met}$ during methionine-restricted and -unrestricted growth of this *metG* mutant indicate a direct correlation between repression of methionyl-tRNA synthetase formation and the extent of aminoacylation of $tRNA^{met}$. These results (Fig. 5-9) suggest that methionyl-tRNA synthetase formation in *S. typhimurium* is regulated by a repression process, as has been reported of *E. coli* (1). Furthermore, all these results are consistent with the idea that methionyl-tRNA, rather than L-methionine, is an essential component for repression control of formation of this synthetase in both *E. coli* and *S. typhimurium*.

DISCUSSION

The data presented in this paper provide evidence that methionyl-tRNA synthetase formation of both *E. coli* and *S. typhimurium* is regulated by a repression process which requires methionyl-tRNA or a derivative thereof as the end-product effector. Thus, these results strengthen our previous findings that methionyl-tRNA synthetase formation is subject to repression control in *E. coli* (1).

For the *E. coli* strain, a mutant isolated as being growth resistant to the methionine analogue, ethionine, was found to possess a methionyl-tRNA synthetase with a reduced affinity for

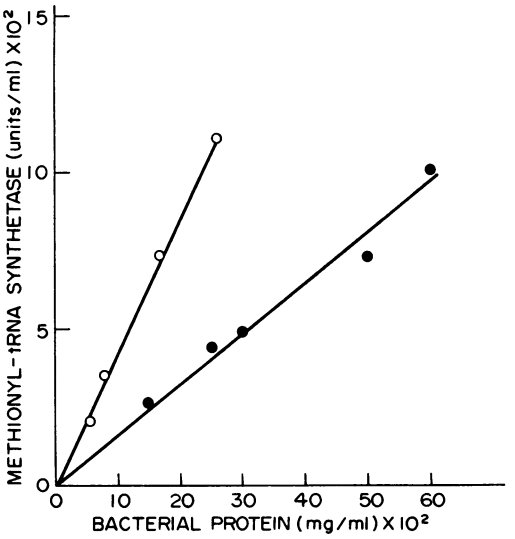

FIG. 7. *Differential rate of formation of methionyl-tRNA synthetase during steady-state growth, SB1365* (O) *was grown exponentially in minimal medium, and SB109* (●) *was grown in minimal medium supplement with 100 μg of methionine per ml. Sampling of cultures, and expression of results are as described in Fig. 2A.*

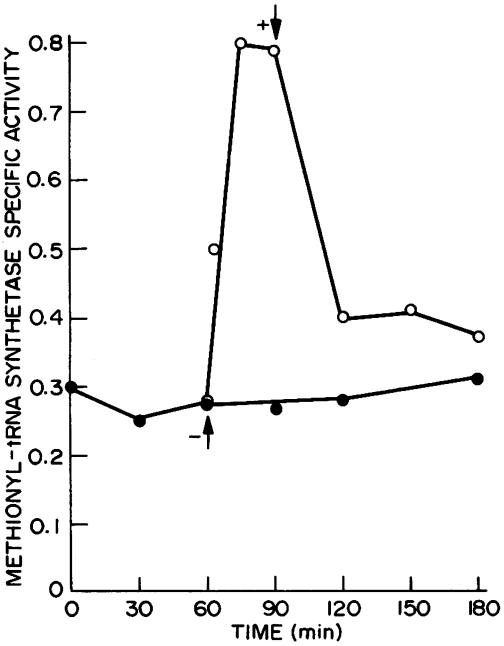

FIG. 8. *Effect of methionine restriction on the specific activity of methionyl-tRNA synthetase of the mutant (SB1365) strain. The cells were grown in unrestricted medium, washed, and transferred to two flasks, one of which contained unrestricted medium and the other methionine-restricted medium. At the time indicated by the arrow, excess methionine was added to the culture. Samples were collected, and activity was determined for the unrestricted (●) and restricted (○) cultures.*

tRNA^{met}. The reduction in in vivo aminoacylation of tRNA^{met} in this mutant is consistent with observed reduction in affinity of the synthetase for tRNA^{met}. For this mutant, the results clearly indicate that the differential rate of methionyl-tRNA synthetase formation was increased several-fold over the rate of synthesis of this enzyme in the ethionine-sensitive, parent strain. Thus, methionyl-tRNA appears to be the physiologically significant unit for repression control of synthesis of this synthetase in *E. coli*.

For the *S. typhimurium* strain, use was made of a methionyl-tRNA synthetase (*metG* mutant) (P. E. Hartman, personal communication). This mutant is essentially a methionine requirer, which results from the considerably reduced affinity of the synthetase for L-methionine. Growth of this mutant in minimal medium in the absence of methionine caused a derepression of methionyl-tRNA synthetase formation. Conversely, during growth of this *metG* mutant in media supplemented with concentrations of L-methionine greater than the K_m, the rate of synthesis of this synthetase was similar to that of the *metG*$^+$ strain grown in methionine-unrestricted medium, and the in vivo aminoacylation of methionine-tRNA increased to a level comparabe to the percent charging observed for the normal strain grown in methionine-unrestricted medium. Furthermore, the density labeling studies provide clear evidence that the derepression involved an increased rate of de novo formation of this synthetase. As mentioned above for the *E. coli* strain, methionyl-tRNA, rather than L-methionine, appears a reasonable choice for the end product effector for repression control of this synthetase in *S. typhimurium*.

The important point in this report is not that the synthetase has a direct role in its own synthesis (i.e., it is not a part of the repressor complex). Rather, we suggest that the synthetase alteration resulted in a derepression of its synthesis due to a reduction in the rate of generating methionyl-tRNA, the catalytic product of the synthetase reaction. Thus, methionyl-tRNA synthetase formation is not constitutive in either of these mutants; rather, under specific growth conditions, a *metG* mutant, in comparison to the *metG*$^+$ strain, would exhibit an elevated rate of methionyl-tRNA synthetase formation.

Based on the results of these studies, it is suggested that methionyl-tRNA is the corepressor for repression control of the cognate synthetase of both *E. coli* and *S. typhimurium*. It should be emphasized that we have not

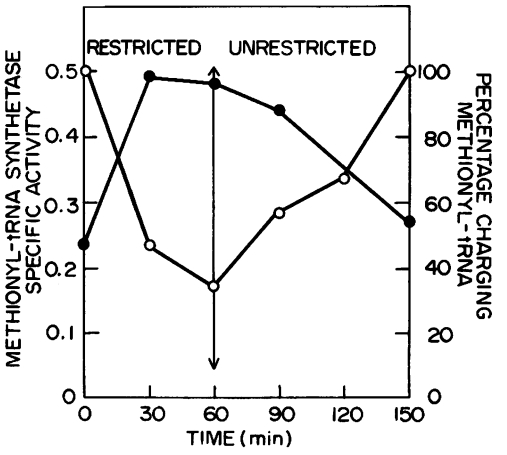

FIG. 9. *Percentage charging of tRNA^{met} and the activity of methionyl-tRNA synthetase during growth in methionine restricted and unrestricted media. Specific activity was determined by the attachment assay, and tRNA was isolated and oxidized with sodium periodate as described in Materials and Methods. Symbols: ●, methionyl-tRNA synthetase activity; ○, percent charging of tRNA^{met}.*

distinguished between formylmethionyl- and methionyl-tRNA. Both species of methionine-tRNA are aminoacylated by the same synthetase, and formylation of methionine occurs after acylation. However, some preliminary results from our laboratory suggest that inhibition of dihydrofolate reductase alters the repression pattern for this synthetase in a *metG*$^+$ strain grown in methionine-unrestricted medium. Furthermore, whereas the studies presented in this report suggest an involvement of methionyl-tRNA in the control of methionyl-tRNA synthetase formation, the reports of other investigators suggest that normal control of methionine biosynthesis is maintained in *metG* mutants of *S. typhimurium* (4). In contrast, we have preliminary evidence that methionine biosynthesis is derepressed in both of the mutants described in this report.

Lastly, the rationale for the isolation of the ethionine-resistant mutant was based on the following assumption. Among cells which had acquired growth resistance to the methionine analogue, L-methionine, some such cells would have become resistant as a function of a mutation in the structural gene for methionyl-tRNA synthetase, resulting in altered activity for this synthetase. The results presented in this report are consistent with strain NP207 being an ethionine-resistant mutant with altered methionyl-tRNA synthetase activity. However, many ethionine-resistant mutants have been shown to be *metK* and *metJ* mutants, in which the mutations occurred in loci that presumably code for regulatory elements for the control of methionine biosynthesis. The results with our mutant have been considered in this respect and we suggest that the possibility of our mutant being a double mutant (i.e., *metK* or *J*, *metG*) is unlikely. This contention is based primarily on the existence of ethionine-sensitive revertants of strain NP207 and on the similarity of response of the ethionine-resistant *E. coli* mutant and the *metG* mutant of *S. typhimurium* to the same physiological conditions. Obviously, this question can be addressed only by genetic analysis of this ethionine-resistant mutant. Thus, we are presently conducting such an analysis, along with the characterization of control of methionine biosynthesis in both of these mutants, of revertants of the ethionine-resistant mutant and with transductants of the *metG* mutant of *S. typhimurium*. We have obtained preliminary evidence that a transductant of the *metG* mutant has normal growth rate in minimal medium and a rate of methionyl-tRNA synthetase formation equal to that of the prototrophic strain, which was used as the phage donor.

ACKNOWLEDGMENTS

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