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Regulation of Synthesis of Methionyl-, Prolyl-, and Threonyl-Transfer Ribonucleic Acid Synthetases of *Escherichia coli*

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Proline- and threonine-restricted growth caused a three- to fourfold derepression of the differential rate of synthesis of the prolyl- and threonyl-transfer ribonucleic acid (tRNA) synthetases, respectively. Similarly, there was approximately a 24-fold derepression in the rate of synthesis of methionyl-tRNA synthetase during methionine restriction. Addition of the respective amino acids to such derepressed cultures resulted in a repression of synthesis of their cognate synthetases. These results support previous findings and further strengthen the idea that the formation of aminoacyl-tRNA synthetases is regulated by some mechanism which is mediated by the cognate amino acids.

In previous studies, repression of synthesis has been reported for six aminoacyl-transfer ribonucleic acid (tRNA) synthetases (4, 5, 6). The results of these investigations suggest that the control of synthesis of these synthetases is by an amino acid-specific repression process which resembles that reported for the control of biosynthesis of the cognate amino acids.

The present study was undertaken to examine further the question of regulation of aminoacyl-tRNA synthetase formation as an effort to learn whether this regulatory process can be generally applied to aminoacyl-tRNA synthetase. In this report, we present evidence that the rates of synthesis of methionyl-, prolyl-, and threonyl-tRNA synthetases are regulated by a repression process, which appears to be mediated by the cognate amino acids.

MATERIALS AND METHODS

Organisms. Three strains of *Escherichia coli* were used in this study. Strain AB1048 is a multiple auxotroph requiring thiamine, histidine, proline, isoleucine, and valine. Strain AB1132 is a multiple auxotroph for the amino acids leucine, isoleucine, histidine, proline, and methionine. Both strains were obtained from E. Adelberg. Strain CW 7 is a threonine auxotroph maintained in our laboratory.

Media and method of cultivation. The minimal medium used in this study was the basal salts solution described by Fraenkel and Neidhardt (2). This solution was supplemented with 0.2% $(NH_4)_2SO_4$ and 0.4% glucose as nitrogen and carbon sources, respectively. The amino acids, methionine, proline, and threonine, were supplied to the cultures at concentrations of 10 to 100 μg/ml. In some experiments, these amino acids were made limiting by using 10 to 15 μg of glycyl-DL-amino acid per ml instead of the respective amino acid. All other required amino acids were supplied at a final concentration of 100 μg/ml and were the L-isomers. The deuterium oxide medium used in this study was prepared and used as described by McGinnis and Williams (4). 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 medium of the same composition prior to the initiation of each experiment. For the experiments employing deuterium oxide medium, all experimental details were as described by McGinnis and Williams (4). Growth was measured by an increase in optical density at 420 nm with a 1-cm light path in a Zeiss PMQ II or Hitachi-Perkin-Elmer, model 101, spectrophotometer.

Preparation of cell extracts. Cells were subject to sonic treatment with a Biosonik III sonifier as described by Chrispeels et al. (1). The protein content was determined colorimetrically by the methods of Lowry et al. (3).

Enzyme measurement. The activity of the three aminoacyl-tRNA synthetases was determined by the ^{14}C-labeled amino acid attachment assay system as described by Chrispeels et al. (1). For each assay, 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 hr. For all differential plots of the results, the rate of synthesis was determined from the slope of the curves.

Measurement of the de novo rate of enzyme synthesis. All procedures were as described by Williams and Neidhardt (6).

Chemicals. Uniformly labeled L-[^{14}C] amino acids were obtained from New England Nuclear Corp., Boston, Mass. K-12 tRNA was purchased from General Biochemicals Corp., Chagrin Falls, Ohio. Cesium chloride was obtained from Pierce Chemical Co., Rockford, Ill., and glycyl-DL-amino acids were from Mann Research Laboratory, New York.

RESULTS

Regulation of synthesis of methionyl-tRNA synthetase. The cells were grown exponentially in unrestricted medium, washed, and then grown with methionine limitation. The growth of strain AB1132, a methionine auxotroph, in unrestricted medium and in media containing various concentrations of methionine is shown in Fig. 1. As can be seen in Fig. 2, upon the transfer from unrestricted to limiting methionine (5 μg/ml), there was an increase in the specific activity of methionyl-tRNA synthetase from 0.2 to 9.5 units/mg of protein. After this initial increase, the activity decreased and was maintained at a level of approximately 10-fold above the specific activity of the unrestricted culture grown with excess methionine (Fig. 2). In contrast, the level of this enzyme in the unrestricted culture was essentially unchanged for the duration of the experiment (Fig. 2). A differential plot of these results revealed that, during methionine-restricted growth, the rate of synthesis of methionyl-tRNA synthetase was about 20-fold greater than that of the unrestricted culture (Fig. 3).

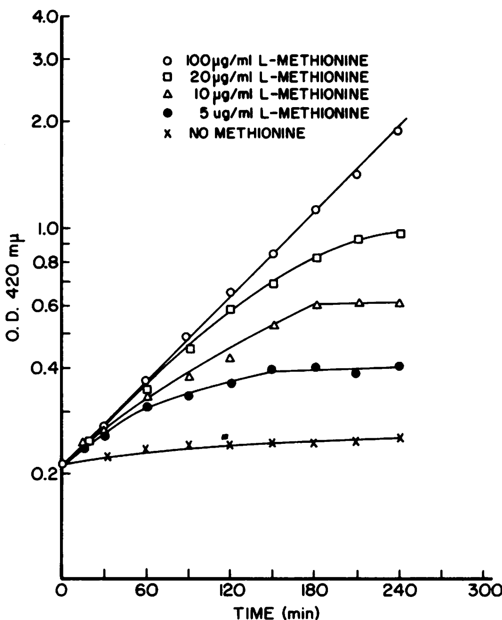

FIG. 1. *Effect of methionine restriction on the growth of E. coli strain AB1132. The cells were grown exponentially in unrestricted medium, washed twice with minimal medium, and transferred to flasks containing different amounts of methionine. Growth was determined for cells growing in minimal medium supplemented with 100 μg/ml, 20 μg/ml, 10 μg/ml, 5 μg/ml, and no methionine.*

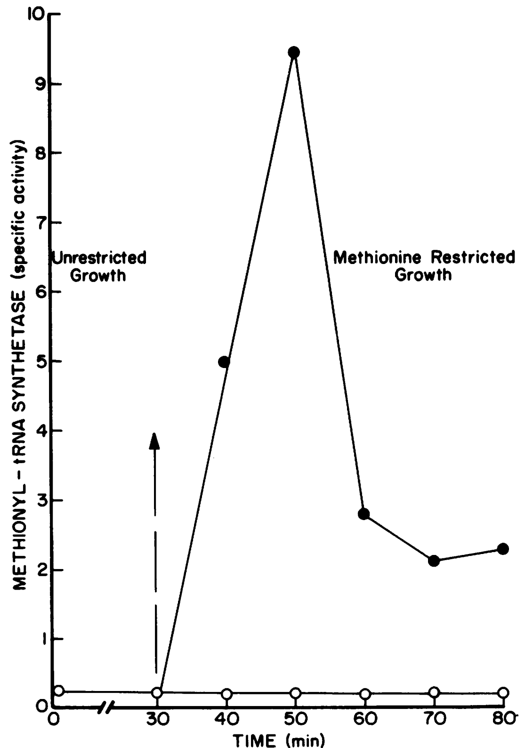

FIG. 2. *Effect of methionine restriction on the specific activity of methionyl-tRNA synthetase. The cells were grown exponentially in unrestricted medium, washed twice, and at the time indicated by the arrow transferred to two flasks, one of which contained unrestricted medium and the other methionine-limiting medium (5 μg/ml). Samples were collected and the activity was determined for the unrestricted (O) and methionine-limited (●) cultures.*

To ascertain whether the differences in activity (Fig. 2) represented a change in the de novo rate of synthesis of methionyl-tRNA synthetase, a density shift experiment was performed as described above with deuterium oxide. The cells were grown in unrestricted deuterium oxide medium, washed twice with water medium, and transferred to methionine-restricted water medium. Figure 4 shows the fractionation, by CsCl centrifugation, of methionyl-tRNA synthetase into heavy (pre-existing) and light (newly synthesized) bands in samples taken at zero, 50, and 100% mass in-

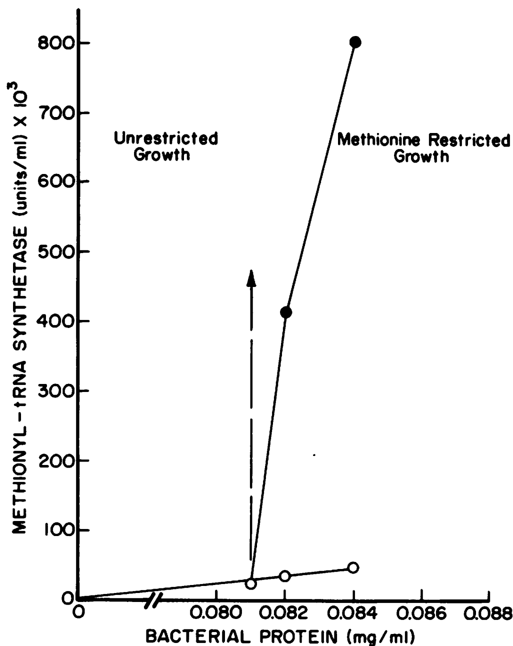

FIG. 3. *Effect of methionine restriction on the differential rate of formation of methionyl-tRNA synthetase. Experimental conditions are those described for Fig. 2. The results are expressed as enzyme units per milliliter of culture as a function of total protein per milliliter of culture for the unrestricted* (O) *and methionine-restricted* (●) *cultures. The shift occurred at the time indicated by the arrow.*

creases after the density shift. The light enzyme units present in the culture at 50 and 100% mass increases were approximately twofold greater than that predicted from the apparent rate before methionine restriction (Fig. 4, panels B and C). The predicted amount of enzyme after the density shift is based on the rate of synthesis before methionine restriction and an unchanged rate of synthesis after the transfer to methionine-restricted medium. However, the amount of heavy enzyme units present after one mass doubling was less than the predicted amount, which may have been due to some type of destructive process or instability, or both, during centrifugation in CsCl. Despite this observation, it is clear from these results that the increase in specific activity of this synthetase during methionine restriction was due to a derepression of the de novo rate of synthesis of this enzyme (Fig. 4). These data were further analyzed as described earlier (4), and a differential plot of the results shown in Fig. 4 indicates a 2.5-fold increase in the rate of synthesis of this synthetase during methionine-restricted growth, as determined by density labeling (Fig. 5). As a control, a change in the density of the medium without manipulation of the methionine supply to the cells had no effect on the rate of synthesis of this enzyme.

Regulation of synthesis of prolyl-tRNA synthetase. Cells of strain AB1048, auxotrophic for proline, were grown in unrestricted medium, washed, and transferred to proline-limiting medium (15 μg of glycyl-DL-proline per ml). As shown in Fig. 6, upon proline restriction, there was a 2.5-fold increase in the specific activity of prolyl-tRNA synthetase. Proline restriction has no effect on the levels of valyl- and leucyl-tRNA synthetase activities (Fig. 6). Addition of proline to the derepressed (proline-restricted) culture caused a repression of synthesis of this enzyme (Fig. 6). A differential plot of the data of Fig. 6 indicates that the rate of formation of prolyl-tRNA synthetase was derepressed fourfold above the rate observed for the unrestricted culture (Fig. 7). Furthermore, proline repressed the differential

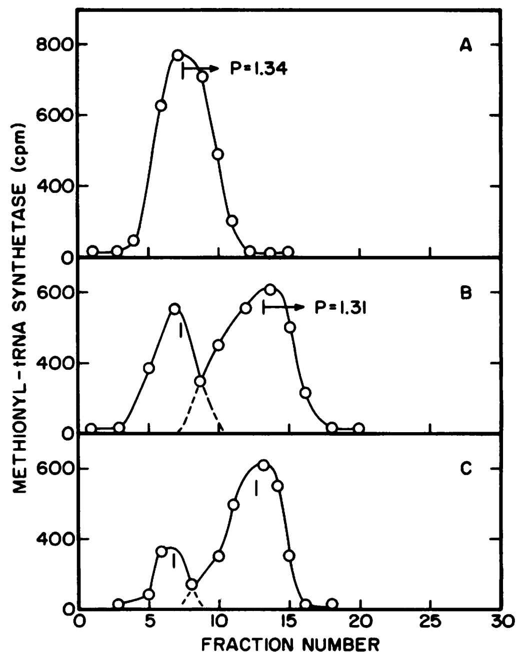

FIG. 4. *Banding in CsCl gradients of methionyl-tRNA synthetase of strain AB1132, grown with methionine limitation after a shift from deuterium oxide to water medium. The three panels showing banding of enzyme in sample A (80% deuterium oxide), B (50% mass increase), and C (100% mass increase) after the shift to water medium. The activity of each fraction is shown as counts per minute of* L-(^{14}C) *methionine attached to tRNA under standard assay conditions; ρ represents the density of the gradients, the bottom of which is to the left of the figure.*

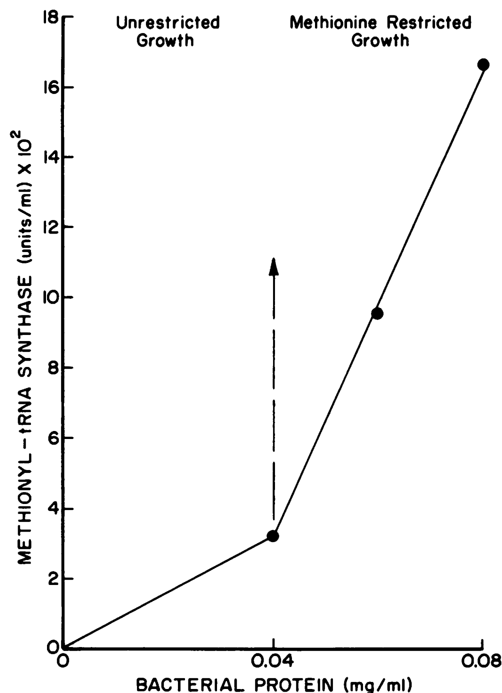

FIG. 5. *Differential rate of formation of methionyl-tRNA synthetase as determined by density labeling. These results are calculated from the rates of synthesis measured in Fig. 4. The data are expressed as total enzyme units per milliliter of culture as a function of total protein per milliliter of culture. The shift from deuterium oxide-unrestricted medium to water-methionine limitation occurred at the time indicated by the arrow.*

rate of formation of this enzyme (Fig. 7). Manipulation of the proline supply to the cells had no effect on the differential rate of formation of the valyl-tRNA synthetase (Fig. 7).

To examine further the synthesis of prolyl-tRNA synthetase, a density shift experiment was performed with deuterium oxide-water as described above. The cells were grown in unrestricted deuterium oxide medium, washed twice with water medium, and transferred to water medium containing 5 μg of glycyl-DL-proline per ml and to another flask of the same medium supplemented with 15 μg of glycyl-DL-proline per ml. Figure 8 shows the fractionation, by CsCl centrifugation, of the synthetase into heavy (pre-existing) and light (newly synthesized) bands. Samples were taken at zero time, 50% mass increase, and 100% mass increase after the density shift; the enzyme units (heavy and light) were determined from the areas under each curve. The results shown in Fig. 8 indicate that the heavy enzyme units present after a 50% mass increase in proline-restricted medium had been reduced to approximately one-half of the original (zero time) amount. However, the light enzyme units present after a 50 and 100% mass increase had increased three to four times above that predicted from the rate of synthesis before proline restriction (Fig. 8). The results also indicate that the cells grown with 15 μg of glycyl-DL-proline per ml exhibited more enzyme units at the 50% mass increase sample than the cells grown with 5 μg of glycyl-DL-proline per ml (Fig. 8), The latter result suggests that a finite amount of proline is necessary for the in vivo stabilization of the enzyme activity. The differential rate of formation of prolyl-tRNA synthetase, as determined by density labeling, was found to be three- to fourfold greater than the rate of synthesis during unrestricted growth of the cells (Fig. 9). Significantly, a transfer from heavy to light medium without proline restriction resulted in a rate of synthesis of this enzyme proportionate with the rate of synthesis of total proteins.

Regulation of synthesis of threonyl-tRNA synthetase. For these studies, strain CW-7, a

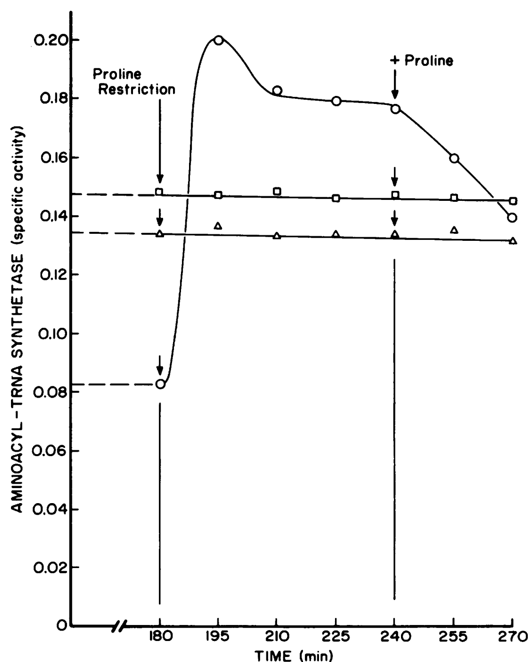

FIG. 6. *Effect of proline restriction on the specific activities of prolyl-, valyl-, and leucyl-tRNA synthetases. The cells were grown in unrestricted medium, washed, and transferred to proline-limiting medium (15 μg of glycyl-DL-proline per ml). The activity was determined for prolyl- (○), valyl- (□), and leucyl- (△) tRNA synthetases. Proline was removed or added at the time indicated by the arrow.*

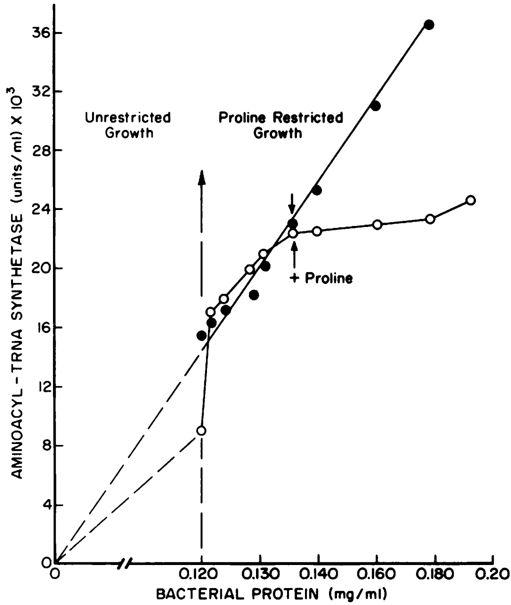

FIG. 7. *Effect of proline restriction on the differential rate of formation of prolyl- and valyl-tRNA synthetases. Experimental conditions are those described for Fig. 6. The cells were transferred to proline-limiting and proline-excess media at the times indicated by the arrows. The results are expressed as units per milliliter as a function of total protein per milliliter of culture for prolyl- (O) and valyl- (●) tRNA synthetases.*

threonine auxotroph, was grown exponentially in unrestricted medium and transferred to threonine-limiting medium. As can be seen in Fig. 10, threonine restriction caused a threefold increase in the specific activity of this synthetase. On the other hand, the unrestricted culture maintained essentially the same specific activity for this synthetase for the duration of the experiment. As observed for methionyl- and prolyl-tRNA synthetases, these results suggest that threonine restriction caused a derepression of the rate of formation of threonyl-tRNA synthetase (Fig. 10).

DISCUSSION

The results presented in this paper provide evidence that synthesis of methionyl-, prolyl-, and threonyl-tRNA synthetases is regulated by a repression process specifically affected by the cognate amino acid.

Cells of *E. coli* grown with limiting amounts of the amino acids methionine, proline, and threonine exhibited derepressed levels of methionyl-, prolyl-, and threonyl-tRNA synthetases, respectively. However, the rate of synthesis of methionyl-tRNA synthetase was derepressed about 20-fold during methionine-restricted growth, whereas the rate of synthesis of prolyl- and threonyl-tRNA synthetases was derepressed three- to fourfold during growth restriction by the respective amino acid. These results could be considered from the point of view that the rate of formation of aminoacyl-tRNA synthetases is related to the frequency of use of the specific amino acid in protein synthesis. On the other hand, the differences in the extent of derepression could simply be

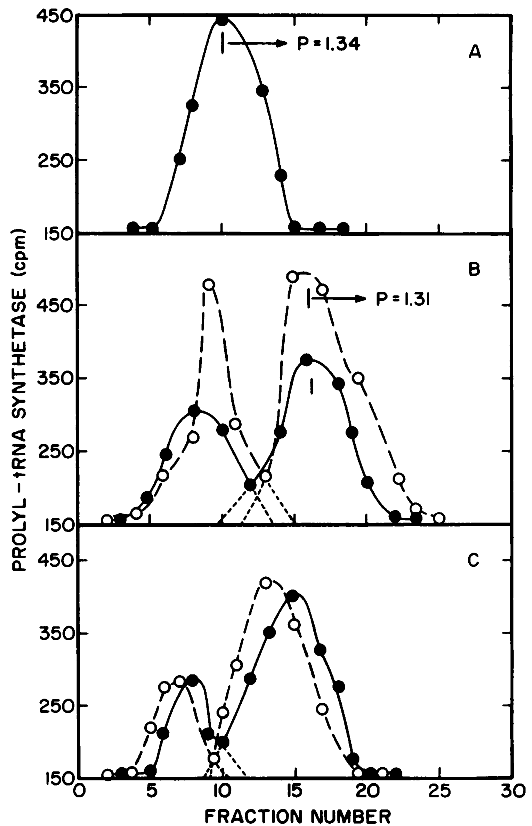

FIG. 8. *Banding in CsCl gradients of prolyl-tRNA synthetase of cells grown with proline restriction after a shift from deuterium oxide to water medium. The cells were grown in deuterium oxide-unrestricted medium and transferred to two different proline-restricted-water medium flasks. A, enzyme units of the zero time sample (80% deuterium oxide); B and C, enzyme present after 50% (B) and 100% (C) increases in mass after shift to water medium for the cells grown with 15 μg/ml (O) and 5 μg/ml (●) of glycyl-DL-proline; ρ represents the density of the gradient, the bottom of which is to the left of the figure.*

fortuitous. Significantly, addition of the respective amino acid to the derepressed cultures caused a decreased rate of synthesis (repression) of the corresponding synthetase.

In further examining the apparent derepression of synthesis of these synthetases, it was of interest to determine whether this change in specific activity was the result of a different rate of formation or the reflection of differences in activities of these synthetases. Consistent with the findings of Williams and Neidhardt (6) and McGinnis and Williams (4), the density labeling data provided evidence that the de novo rate of formation of methionyl- and prolyl-tRNA synthetases was derepressed during restricted growth of cells by the cognate amino acid. Thus, the rate of synthesis, rather than the activity, of these synthetases was specifically affected by manipulation of the supply of the cognate amino acid to the cells.

The data in this report strengthen the previous findings of regulation of synthesis of isoleucyl-, phenylalanyl-, arginyl-, histidyl-, valyl-, and leucyl-tRNA synthetases (4–6). Including the results of the present report, regulation of synthesis by a repression process, apparently mediated by the respective amino acid, has been observed for 9 of the 20 aminoacyl-tRNA synthetases.

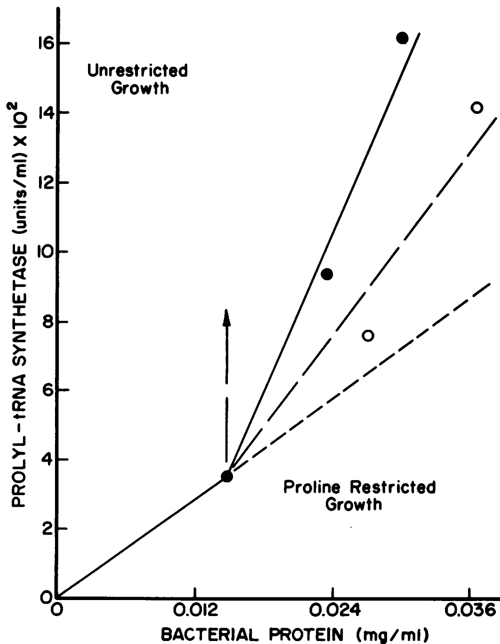

FIG. 9. *Differential rate of formation of prolyl-tRNA synthetase as determined by density labeling. These results were calculated from the rates of synthesis measured in Fig. 8. The data are expressed as enzyme units per milliliter as a function of total protein per milliliter of culture. The shift from deuterium-oxide unrestricted medium to water-methionine [15 μg/ml (O) and 5 μg/ml (●)] limitation occurred at the time indicated by the arrow.*

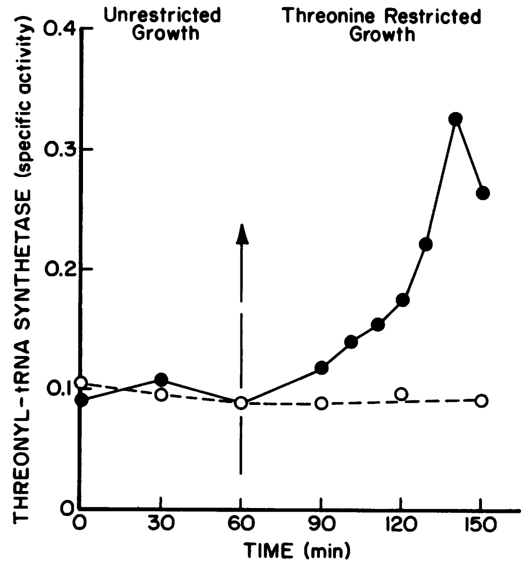

FIG. 10. *Effect of threonine restriction on the specific activity of threonyl-tRNA synthetase. The cells were grown exponentially in minimal medium supplemented with excess threonine, washed, and at the time indicated by the arrow transferred to unrestricted and threonine-limiting media. Samples were collected and assayed for threonyl-tRNA synthetase activity of the unrestricted* (O) *and the threonine-limited* (●) *cultures.*

ACKNOWLEDGMENTS

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