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Regulation of Synthesis of the Aminoacyl-Transfer Ribonucleic Acid Synthetases for the Branched-Chain Amino Acids of *Escherichia coli*

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The regulation of synthesis of valyl-, leucyl-, and isoleucyl-transfer ribonucleic acid (tRNA) synthetases was examined in strains of *Escherichia coli* and *Salmonella typhimurium*. When valine and isoleucine were limiting growth, the rate of formation of valyl-tRNA synthetase was derepressed about sixfold; addition of these amino acids caused repression of synthesis of this enzyme. The rate of synthesis of the isoleucyl- and leucyl-tRNA synthetases was derepressed only during growth restriction by the cognate amino acid. Restoration of the respective amino acid to these derepressed cultures caused repression of synthesis of the aminoacyl-tRNA synthetase, despite the resumption of the wild-type growth rate.

In several recent studies, repression of synthesis has been demonstrated for several aminoacyl-transfer ribonucleic acid (tRNA) synthetases (7, 10). Nass and Neidhardt (7) have reported the synthesis of isoleucyl- and phenylalanyl-tRNA synthetase could be derepressed two- to threefold over the normal by growing cells with restriction of the relevant amino acid. Later, Williams and Neidhardt (10) reported that derepression of synthesis of arginyl- and histidyl-tRNA synthetase occurred during restricted growth of auxotrophs and bradytrophs by the cognate amino acid. By use of density labeling with deuterium oxide, it was possible to show that derepression of synthesis of these two synthetases was correlated with an increased rate of de novo synthesis of the enzymatically active proteins (10).

The present study was undertaken to compare the regulation of formation of the synthetases for the branched-chain amino acids and to learn whether there are different mechanisms for regulating synthesis of different aminoacyl-tRNA synthetases. In this paper, we report evidence that the rates of synthesis of valyl-tRNA synthetase [EC 6.1.1,9, valine: soluble RNA (sRNA) ligase, adenosine monophosphate (AMP)], isoleucyl-tRNA synthetase (EC 6.1.1,5, isoleucine: sRNA ligase, AMP), and leucyl-tRNA synthetase (EC 6.1.1,4, leucine: sRNA ligase, AMP) are regulated by a mechanism resembling repression. We also report evidence that synthesis of valyl-tRNA synthetase is regulated by both valine and isoleucine in a multivalent manner, whereas the rate of formation of leucyl- and isoleucyl-tRNA synthetases is specifically affected by the supply of the respective amino acid to the cells.

A preliminary report of some of these results has appeared (E. McGinnis and L. S. Williams, Bacteriol. Proc., p. 157, 1971).

MATERIALS AND METHODS

Organisms. Three strains of *Escherichia coli* and one of *Salmonella typhimurium* were used in this study. Strain M4862-H-5 (obtained from M. Freundlich) is an isoleucine, valine, and leucine auxotroph of *E. coli* W. Strain NP2 (obtained from F. C. Neidhardt) is a valine-sensitive prototrophic strain of *E. coli* maintained in our laboratory. Strain AB1132 (obtained from E. Adelberg) is a multiple auxotroph of *E. coli* requiring histidine, leucine, methionine, proline, and thiamine. Strain 2428 (obtained from H. E. Umbarger) is an isoleucine and valine auxotroph of *S. typhimurium*.

Media and methods of cultivation. The minimal medium used was the basal salts solution described by Fraenkel and Neidhardt (4) supplemented with 0.4% glucose and 0.2% ammonium sulfate as carbon and nitrogen sources, respectively. When added, amino acids were the L-isomers and were used at 50 μg/ml unless otherwise noted. Valine, leucine, and isoleucine were made limiting by using 10 to 20 μg of DL-glycyl-amino acid per ml instead of the respective amino acid. Deuterium oxide medium was prepared by using deuterium oxide (80%) instead of water. 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 deuterium oxide medium, the cells were pregrown for several days in the heavy medium. To remove amino acid supplements and change from deuterium oxide to water media, cultures were chilled rapidly, centrifuged under sterile conditions, washed twice with medium of desired composition, and the cells were then resuspended in prewarmed medium. 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 subjected to sonic treatment with a Branson sonifier as described by Chrispeels et al. (3). The protein content of the extracts was determined by the method of Lowry et al. (6).

Enzyme measurement. The activity of the various aminoacyl-tRNA synthetases was measured by the ^{14}C-labeled amino acid attachment assay as described by Chrispeels et al. (3), with the exception that incubation was at 30 C for 5 min. For all assays, the values reported are corrected for a minus adenosine triphosphate blank and are the averages of at least three determinations. One unit of activity was defined as that which formed 1 μmole of product per hr. Specific activity was expressed as units per milligram of protein.

Centrifugation in cesium chloride. All procedures were as described by Williams and Neidhardt (10). The equilibrium centrifugation was performed in a Spinco model SW 50.1 rotor by using a Spinco-Beckman model L4 ultracentrifuge.

Measurement of the de novo rate of enzyme synthesis. The true rate of synthesis of valyl-tRNA synthetase was determined by methods already described by Williams and Neidhardt (10).

Unrestricted and restricted growth of cells. The results of previous studies (Williams and Neidhardt, and Nass and Neidhardt) provided the experimental rationale for the present study. In the earlier studies, it was shown that the differential rate of formation of several aminoacyl-tRNA synthetases was increased only when the intracellular level of amino acids was below that maintained by a prototrophic cell growing in minimal medium (10). Therefore, for our studies, we made use of the dipeptides, glycyl-DL-valine, glycyl-DL-leucine, and glycyl-DL-isoleucine, to impose specific amino acid-restricted growth of the auxotrophs. In addition, short-term growth restriction of a leucine auxotroph was accomplished by transferring cells from unrestricted to leucine-restricted media without washing the cells to remove the growth factors.

E. coli strain M4862-H-5, an isoleucine, valine, and leucine auxotroph, was grown for several generations in minimal medium supplemented with excess valine, leucine, and isoleucine. The cells were washed twice with minimal medium and transferred to several flasks of minimal media containing 5 to 20 μg of a specific dipeptide (glycyl-DL-valine, glycyl-DL-leucine, or glycyl-DL-isoleucine) per ml, with the other two required amino acids in excess. These preliminary growth studies were conducted to ascertain that the specific dipeptide caused restriction of growth (about 35 to 50% of unrestricted growth) and to determine the duration of the invariant rate of restricted growth. A concentration of 15 μg/ml was found to be suitable for the experiments.

Source of chemicals. *E. coli* K-12 tRNA was purchased from General Biochemical Corp. (Chagrin Falls, Ohio). Uniformly labeled L-[^{14}C] amino acids were obtained from New England Nuclear Corporation (Boston, Mass.). Chloramphenicol was purchased from Sigma Chemical Co. (St. Louis, Mo.), and cesium chloride from Pierce Chemical Co. (Rockford, Ill.), and glycyl-DL-leucine, glycyl-DL-valine, and glycyl-DL-isoleucine were from Mann Research Laboratory (New York).

RESULTS

Regulation of synthesis of valyl-tRNA synthetase. The cells were grown exponentially in unrestricted medium, washed, and grown with limiting valine, limiting isoleucine, and limiting leucine. The growth of strain M4862-H-5 in isoleucine-limiting, valine-limiting, leucine-limiting, and unrestricted media is shown in Fig. 1. As shown in Fig. 2, upon the shift from unrestricted to the valine-limited culture, there was a five- to sixfold derepression in the rate of formation of valyl-tRNA synthetase. The addition of valine to this culture caused a repression of synthesis of the enzyme. Interestingly, in the isoleucine-limited culture, a similar derepression of the differential rate of formation of valyl-tRNA synthetase occurred; and there was a reduction in the rate of synthesis upon the addition of isoleucine to this culture (Fig. 2). The rate of synthesis of valyl-tRNA synthetase was unaffected, however, by manipulation of the leucine supply to the cells (Fig. 1).

The results shown in Fig. 2 indicate that synthesis of valyl-tRNA synthetase was regulated in

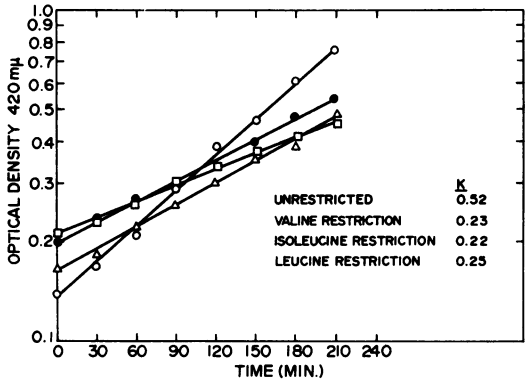

FIG. 1. *Effect of valine, isoleucine, and leucine restrictions on growth of E. coli strain M4862-H-5. Cells were grown exponentially in minimal medium supplemented with excess valine, isoleucine, and leucine. The cells were washed twice with minimal medium and transferred to three separate flasks, each containing 15 μg of a specific glycyl-DL branched-chain amino acid per ml. Growth rate was determined for the cells growing in unrestricted* (○), *valine-limiting* (△), *isoleucine-limiting* (□), *and leucine-limiting* (●) *media.*

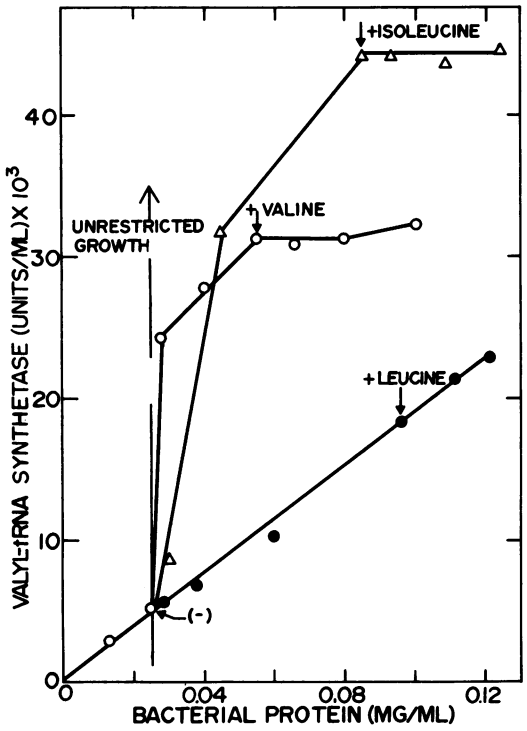

FIG. 2. *Effect of valine, leucine, and isoleucine restriction on the differential rate of formation of valyl-tRNA synthetase in an isoleucine, valine, and leucine auxotroph. A culture of strain M4862 H-5 in balanced-growth minimal medium supplemented with valine (100 μg/ml), leucine (50 μg/ml), and isoleucine (50 μg/ml) was chilled rapidly, and the cells collected by centrifugation were washed twice with minimal medium and then incubated in minimal medium with glycyl-D L-valine (17.5 μg/ml), limiting glycyl-D L-isoleucine (17.5 μg/ml), and glycyl-D L-leucine (15 μg/ml) as separate cultures. Amino acids were removed from the unrestricted culture or added to the restricted culture at the time indicated by the arrows. Samples taken at intervals throughout the three growth periods were used to assay for valyl-tRNA synthetase activity in the valine-limiting (○), the isoleucine-limiting (△), and the leucine-limiting (●) media. Results are plotted as enzyme units per milliliter of culture as a function of total protein per milliliter of culture.*

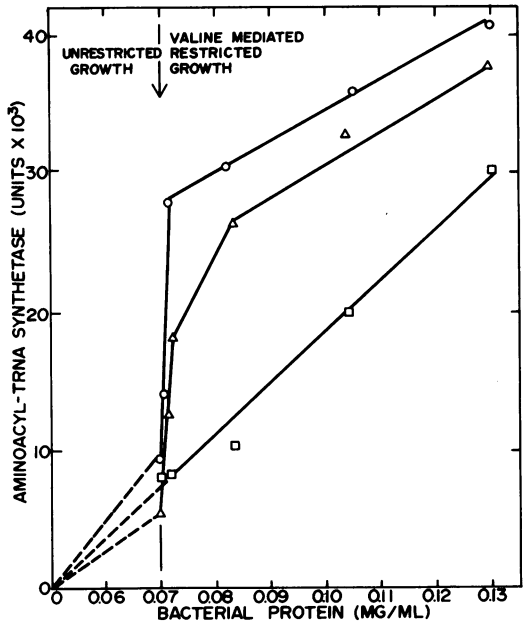

FIG. 3. *Effect of isoleucine restriction on the differential rate of formation of valyl-, isoleucyl- and leucyl-tRNA synthetases. Strain NP2 (valine-sensitive prototroph) was grown exponentially in minimal medium and received L-valine (40 μg/ml) at the time indicated by the arrow. The activity was determined for valyl-(○), isoleucyl-(△), and leucyl-(□) tRNA synthetases. Data are plotted as described for Fig. 2.*

response to both valine and isoleucine. These observations suggest that the control was multivalent in nature, as has been shown for the valine and isoleucine biosynthetic enzymes (5). To induce an isoleucine restriction in a alternative way, use was made of strain NP2, the valine-sensitive wild-type strain.

Strain NP2 was grown for several generations in minimal medium, and then the culture was supplemented with L-valine (40 μg/ml). As shown in Fig. 3, upon the initiation of this valine-mediated isoleucine restriction, there was a derepression of the rate of synthesis of both the valyl- and isoleucyl-tRNA synthetases. However, isoleucine restriction had no affect on the rate of formation of leucyl-tRNA synthetase. These results (Fig. 3) clearly show that the rate of formation of valyl-tRNA synthetase is affected by the isoleucine supply to the cells. We considered the possibility that these results (Fig. 3) might have been intrinsically related to the valine sensitivity character of the strain employed. Therefore, a similar experiment was conducted by using a *S. typhimurium* strain that was auxotrophic for valine and isoleucine. As shown in Fig. 4, upon a shift from unrestricted to isoleucine-restricted growth of this strain, there was about an eightfold derepression of the differential rate of synthesis of both the valyl- and isoleucyl-tRNA synthetases. Addition of isoleucine to the derepressed culture repressed the synthesis of both enzymes. As observed for the two *E. coli* strains, the rate of formation of leucyl-tRNA synthetase of *S. typhimurium* was not affected by a change in supply of isoleucine to the cells.

Regulation of synthesis of isoleucyl-tRNA synthetase. The above results (Fig. 3 and 4) indicate

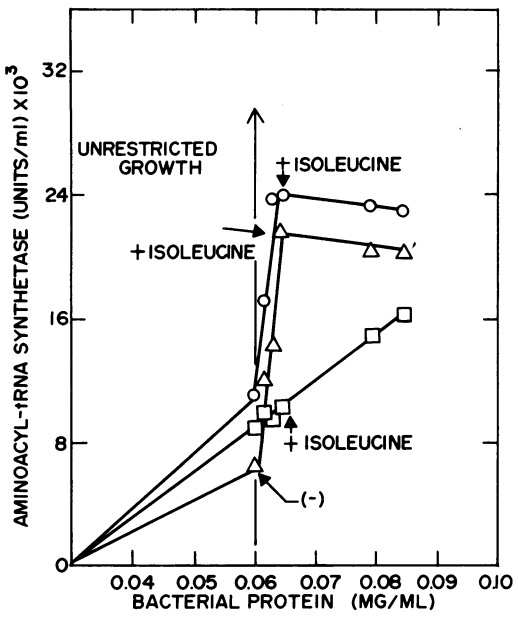

FIG. 4. *Effect of isoleucine restriction on the differential rate of formation of isoleucyl-, valyl-, and leucyl-tRNA synthetases. Salmonella typhimurium strain 2428 was grown exponentially in minimal medium with excess valine and isoleucine. At the time indicated by the arrow, the cells were washed twice and resuspended in minimal medium with limiting isoleucine (17.5 μg ml). The activity was determined for valyl- (○), isoleucyl- (△), and leucyl- (□) tRNA synthetases. Results are expressed as enzyme units per milliliter of culture as a function of total protein per milliliter of culture.*

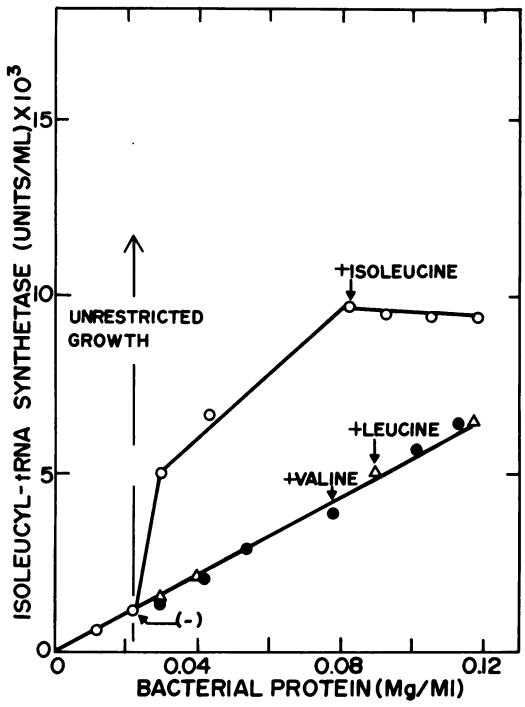

FIG. 5. *Effect of isoleucine, valine, and leucine restriction on the differential rate of formation of isoleucyl-tRNA synthetase. Experimental conditions are as described for Fig. 2. The activity was determined for isoleucyl-tRNA synthetase in the isoleucine-limited culture (○), the valine-limited culture (●), and the leucine-limited culture (△).*

that, in both *E. coli* and *S. typhimurium*, isoleucine restriction caused an increase in the differential rate of formation of isoleucyl-tRNA synthetase. Since isoleucine restriction affected the rate of synthesis of valyl-tRNA synthetase (Fig. 2, 3, and 4), an obvious question is whether there is any effect of valine- and leucine-restricted growth on the rate of synthesis of isoleucyl-tRNA synthetase.

Strain H-5 was grown for two generations in the unrestricted medium and then shifted to isoleucine-restricted medium (17.5 μg of isoleucine per ml). The results of these experiments are shown in Fig. 5. It can be observed that isoleucine restriction resulted in an eightfold derepression of the differential rate of formation of isoleucyl-tRNA synthetase. Addition of excess isoleucine repressed the synthesis of this enzyme (Fig. 5). Significantly, neither valine nor leucine restriction caused a measurable change in the rate of synthesis of the enzyme (Fig. 5). In contrast to valyl-tRNA synthetase, the rate of synthesis of isoleucyl-tRNA synthetase was affected only by manipulation of the supply of the cognate amino acid, isoleucine, to the cells.

Regulation of synthesis of leucyl-tRNA synthetase. The results reported above (Fig. 2 to 5) indicate that valine and isoleucine had no affect on the rate of synthesis of leucyl-tRNA synthetase. The regulation of leucyl-tRNA synthetase formation was examined in more detail by growing strain M4862-H-5 in three separate flasks containing limiting leucine, valine, and isoleucine. As shown in Fig. 6, only leucine restriction caused a derepression of the differential rate of formation of leucyl-tRNA synthetase. The restoration of excess leucine to this derepressed culture caused repression of synthesis of this enzyme, whereas the rate of synthesis was unchanged during shifts from unrestricted to valine- and isoleucine-restricted growth.

It was of interest to examine the effect of leucine restriction on the synthesis of the cognate synthetase in another *E. coli* strain. For these experiments, strain AB1132, a leucine auxotroph, was grown with excess leucine and the cells were

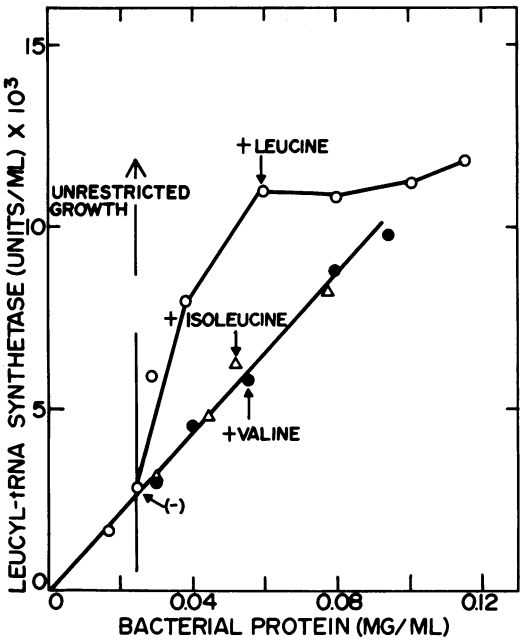

FIG. 6. *Effect of isoleucine, valine, and leucine restriction on the differential rate of formation of leucyl-tRNA synthetase. Experimental conditions are as described for Fig. 2. The symbol designations represent the activity of leucyl-tRNA synthetase in the leucine-limited culture* (○), *the isoleucine-limited culture* (△), *and the valine-limited culture* (●).

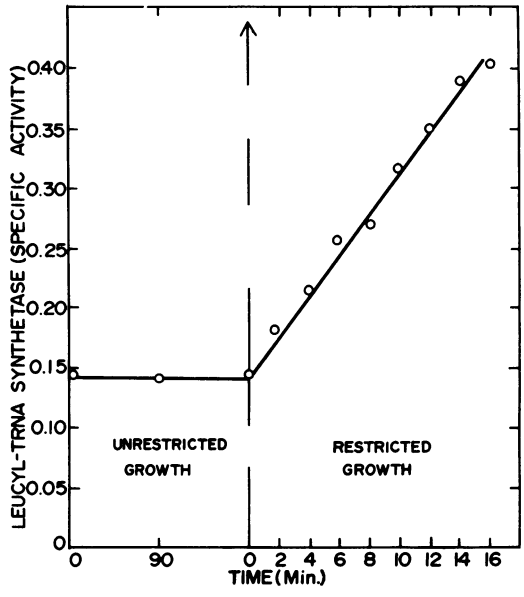

FIG. 7. *Effect of leucine restriction on the specific activity of leucyl-tRNA synthetase of a leucine auxotroph. A culture of strain AB1132 was grown in minimal medium with excess amounts of leucine and all other required growth factors, and the cells were transferred without washing to the fresh medium, which contained no leucine. At the time indicated by the arrow, samples were taken at 2-min intervals for 16 min, and the activity of leucyl-tRNA synthetase was determined as described in Materials and Methods.*

transferred without washing to a medium lacking leucine. As shown in Fig. 7, there was a 2.7-fold increase in the specific activity of leucyl-tRNA synthetase during the first 16 min of leucine deprivation. Samples were taken within the first 20 min of growth to avoid sampling during periods of severe leucine starvation. Early sampling is especially important since Williams and Neidhardt previously observed a loss of arginyl-tRNA synthetase activity during arginine limitation of an auxotroph in a chemostat (10). In fact, as shown in Fig. 8, a similar inactivation of leucyl-tRNA synthetase occurred after 1 hr of leucine starvation of strain AB1132. The activity of this enzyme increased several fold within 30 min, and yet there was an 80% reduction of activity during 2.5 hr of incubation (Fig. 8). When excess leucine was added to the culture, the specific activity increased to the original level, whereas there was very little change in activity during 5 hr of growth in excess leucine (Fig. 8).

Inactivation of synthetase activity. The results described above (Fig. 8) indicate that there was a loss of leucyl-tRNA synthetase activity as the leucine auxotroph approached an apparent state of leucine starvation (i.e., no further increase in optical density). The question of turnover of valyl-tRNA synthetase was examined in strain M4862-H-5, grown with limiting glycyl-DL-valine (2.5 to 20 μg/ml). As shown in Table 1, although 2.5 and 5 μg of glycyl-DL-valine per ml were sufficiently low to allow derepression of valyl-tRNA synthetase, this derepression was followed by loss of activity (46% for 2.5 μg/ml and 38% for 5 μg/ml). A glycyl-DL-valine concentration of 10 μg/ml allowed an increase in specific activity, with little apparent loss of activity, whereas with 20 μg/ml there was less of an increase in specific activity, without any loss of activity (Table 1). Interestingly, for both derepression of synthesis and loss of activity, valine limitation had little, if any, affect on the leucyl- and isoleucyl-tRNA synthetases (Table 1). However, upon starvation for the specific required amino acid, there was a significant reduction in activity (75% for valyl-, 77% for isoleucyl, and 50% for leucyl) of the cognate aminoacyl-tRNA synthetase (Fig. 9).

Rate of de novo synthesis of valyl-tRNA synthetase during restricted growth. In view of the results (Fig. 8 and 9, and Table 1) showing a loss

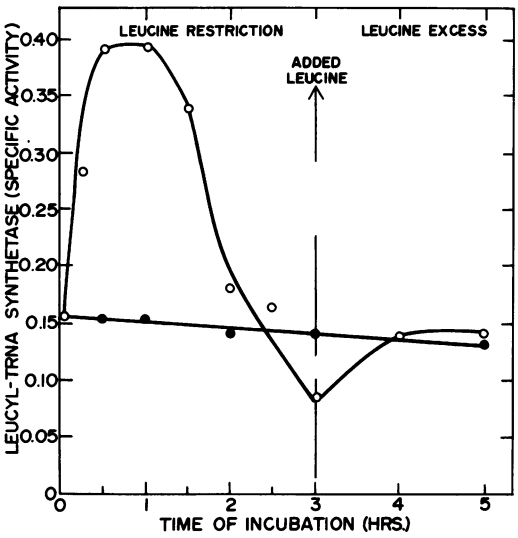

FIG. 8. *Effect of* L-*leucine supply on the specific activity of leucyl-tRNA synthetase of a leucine auxotroph. A culture of strain AB1132 was grown in minimal medium with excess amounts of leucine and other growth factors, and cells were transferred without washing to two flasks, one containing excess leucine and the other no leucine. At the time indicated by the arrow, excess leucine was added to the no-leucine culture, and the activity of leucyl-tRNA synthetase was determined for the no-leucine* (○) *and excess leucine* (●) *cultures.*

TABLE 1. *Effect of valine restriction on synthesis of valyl-, isoleucyl-, and leucyl-transfer ribonucleic acid (tRNA) synthetases of strain M4862 H-5*

Aminoacyl-tRNA synthetase	Glycyl-DL-valine conc (μg/ml)	Specific activity[a] time after shift to valine-restricted media (min)[b]			
		0	30	120	180
Valyl.....	2.5	0.23	0.35	0.31	0.19
Valyl.....	5.0	0.23	0.56	0.53	0.35
Valyl.....	10	0.23	0.50	0.50	0.43
Valyl.....	20	0.23	0.33	0.32	0.31
Isoleucyl.....	2.5	0.110	0.118	0.116	0.112
Isoleucyl.....	5.0	0.110	0.120	0.115	0.115
Isoleucyl.....	10	0.110	0.125	0.114	0.116
Isoleucyl.....	20	0.110	0.120	0.110	0.115
Leucyl.....	2.5	0.150	0.142	0.144	0.147
Leucyl.....	5.0	0.150	0.148	0.150	0.147
Leucyl.....	10	0.150	0.150	0.154	0.146
Leucyl.....	20	0.150	0.154	0.150	0.152

[a] Specific activity is expressed as micromoles of product formed per hour per milligram of protein.

[b] At zero time, the cells of an unrestricted culture were washed twice and resuspended in minimal medium supplemented with the indicated amounts of glycyl-DL-valine. The time equals the sampling time.

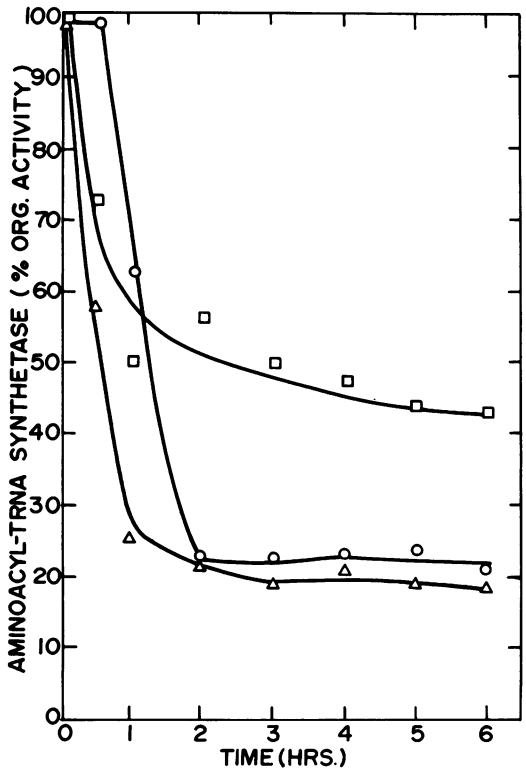

FIG. 9. *Effect of* L-*isoleucine,* L-*valine, and* L-*leucine starvation on in vivo level of the respective aminoacyl-tRNA synthetase. Strain M4862 H-5 was grown in minimal medium supplemented with isoleucine, valine, and leucine. Cells were washed twice with minimal medium and divided into three flasks, one starved of isoleucine, another valine, and another leucine. The activity of valyl-* (○), *isoleucyl-* (△), *and leucyl-* (□) *tRNA synthetases was determined as described in Materials and Methods.*

of synthetase activity under conditions of valine-, leucine-, and isoleucine-restricted growth, it was important to determine whether net change in synthetase activity was due to a different rate of synthesis or a change in preexisting activity. For these experiments, use was made of deuterium oxide and water media to create a density difference between molecules of cells grown under amino acid-restricted and -unrestricted conditions.

The experiments were performed as described above. Strain H-5 was grown in deuterium oxide minimal medium supplemented with excess valine, leucine, and isoleucine and then shifted to water minimal medium supplemented with excess leucine and isoleucine and 15 μg of glycyl-DL-valine per ml. Figure 10 presents the fractionation, by CsCl centrifugation, of valyl-tRNA synthetase into heavy (preexisting) and light (newly

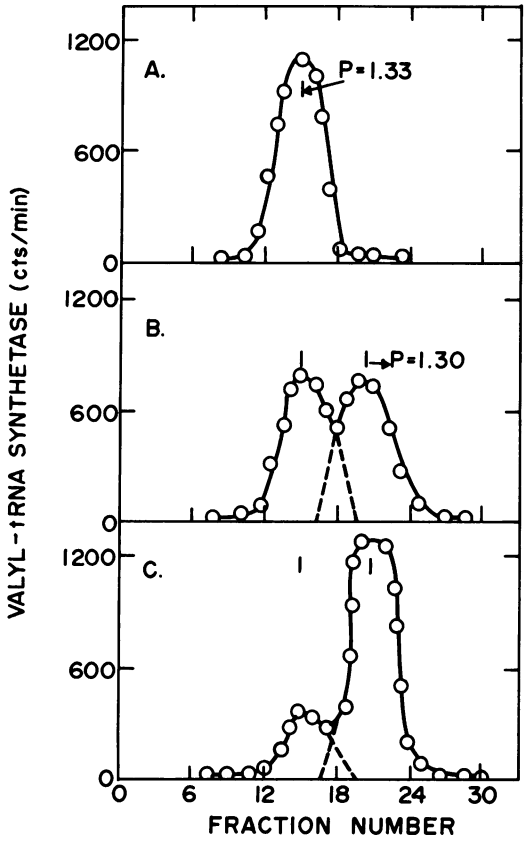

FIG. 10. *Banding in CsCl gradients of valyl-tRNA synthetase of strain M4862 H-5, grown with valine limitation after a shift from deuterium oxide to water medium. The three panels show banding of enzyme in sample A (80% D_2O), sample B (50% mass increase after shift to water medium), and sample C (100% mass increase after shift to water medium). The bottom of the gradient is to the left of each panel. Activity of the fractions is shown as counts per minute of* L-[^{14}C]*-valine attached to tRNA under the standard assay conditions. Results shown in this figure are analyzed further in Table 2.*

synthesized) bands in samples removed at zero time (A), 50% mass increase (B), and 100% mass increase, (C) after the density shift. Tabulations of these results are shown in Table 2, in which it can be observed in column 6 that there was no turnover of preexisting enzyme after one-half mass doubling in valine-limiting medium. However, the heavy enzyme units present after one mass doubling was approximately one-half the predicted amount. (Table 2, Fig. 10C). This result suggests that the enzyme had been reduced to this level by some type of destructive process. On the other hand, the light (newly synthesized) enzyme units present in the culture, at 50 and 100% mass increases, were two to three times that determined from the apparent rate prior to valine restriction (Fig. 10B and C, Table 2). Thus, the increase in specific activity of valyl-tRNA synthetase during valine-limiting growth was due to a derepression of the de novo rate of formation of this enzyme. If one considers the simple assumption that the rate of inactivation is the same for preexisting and newly made enzyme, the true rate of synthesis was approximately twice that observed in these experiments (Fig. 10, Table 2).

A plot of the data tabulated in Table 2 is shown in Fig. 11, in which the derepressed rate of synthesis of valyl-tRNA synthetase was calculated from the rate of appearance of this enzymatically active protein in the culture. The latter results (Fig. 10 and 11, Table 2) indicate the value of the density-labeling method for detection of derepression (increased rate of de novo synthesis) of enzymes. The method also allows delineation between derepressed rate of synthesis and presumably concomitant inactivation of valyl-tRNA synthetase activity. It is reasonable to assume that upon restoration of excess valine to the derepressed culture, the inactivation of activity was halted and repression of synthesis of valyl-tRNA synthetase was initiated (Fig. 2).

However, it is apparent that a part of the reduction in activity was due to inactivation of enzyme activity during centrifugation in cesium chloride, in which the recovery of valyl-tRNA synthetase activity varied from 45 to 60% in different experiments. Furthermore, heavy and light enzyme may have exhibited differential inactivation of activity in these experiments. This question is being examined in more detail in our laboratory.

DISCUSSION

The data presented in this paper provide evidence that synthesis of isoleucyl-, valyl-, and leucyl-tRNA synthetases is regulated by a repression-like mechanism, which responds to specific amino acid limitation.

Valine and isoleucine restrictions of auxotrophs of *E. coli* and *S. typhimurium* derepress the rate of formation of valyl-tRNA synthetase by five- to sixfold over the normal (unrestricted) rate. These changes in rate of synthesis are specific for valine and isoleucine, easily detected, and reproducible. Restoration of valine and isoleucine to the derepressed culture causes an immediate repression of synthesis of the synthetase for at least 50% of a mass doubling, even though the unrestricted rate of growth is resumed. The effects of both valine and isoleucine on the rate of synthesis of valyl-tRNA synthetase

TABLE 2. *Rate of de novo synthesis of valyl-tRNA synthetase of strain M4862 H-5, during valine limitation, as revealed by deuterium labeling*[a]

Sample	Time after density shift	Protein in culture (mg/ml)	Specific activity of extract (units/mg of protein	Protein on gradient (mg)	Heavy[b] enzyme on gradient (units × 10^3)	Light[c] enzyme on gradient (units × 10^3)	Total[d] enzyme in culture (units/ml × 10^3)	Heavy[e] enzyme in culture (units/ml × 10^3)	Light[e] enzyme in culture (units/ml × 10^3)
A	0	0.024	0.195	0.080	6.99	0.00	4.68	4.68	0.00
B	35	0.035	0.585	0.090	5.35	5.31	20.47	(6.89)[f] 10.44	(13.58)[f] 10.03
C	80	0.049	0.560	0.090	1.85	10.68	27.44	(1.14)[f] 4.12	(26.30)[f] 23.32

[a] A culture of strain H-5 was grown for many generations in deuterium oxide medium supplemented with excess amounts of isoleucine, valine, and leucine. At zero time, exponentially growing cells were collected by centrifugation, washed twice, and suspended in water medium with excess isoleucine and leucine, but limiting valine (15 μg of glycyl-DL-valine per ml). Samples were taken at zero time, at 50%, and 100% increases in mass after the shift to valine-limiting water medium. A portion of each extract was analyzed by CsCl centrifugation in the standard way, as shown in Fig. 9.

[b] Obtained by summing the total heavy enzyme units in the CsCl gradients.

[c] Obtained by summing the total light enzyme units in the CsCl gradients.

[d] Calculated by multiplying the specific activity of each extract by the amount of protein (mg/ml) in the culture at the time of sampling.

[e] Amounts of heavy and light enzyme per milliliter of culture were calculated from the value for the total amount of enzyme per milliliter of culture by assuming that the relative amounts of heavy and light enzyme were the same as displayed on the CsCl gradients of Fig. 9.

[f] Amounts of heavy enzyme per milliliter of culture were calculated by multiplying the original (zero time) specific activity (0.195) by the amount of protein per milliliter in the culture at the time of sampling, relative to the heavy-to-light ratio of enzyme displayed in the CsCl gradients.

suggest that regulation of synthesis of this enzyme is multivalent. Thus, the regulation of synthesis of valyl-tRNA synthetase appears to mimic the well-established multivalent repression of the isoleucine and valine biosynthetic enzymes (5). However, this multivalent repression of synthesis of valyl-tRNA synthetase differs from that of the biosynthetic enzymes in the following respects. For the synthetase, the multivalent effect is due to valine and isoleucine alone, and derepression of synthesis is observed only under conditions in which the intracellular levels of the amino acids are below that maintained by a prototrophic cell grown in minimal medium.

The density-labeling data provide evidence that the de novo rate of formation of valyl-tRNA synthetase during valine limitation is greater than the rate under unrestricted growth conditions.

In contrast to valyl-tRNA synthetase, only isoleucine restriction causes a derepression of the rate of synthesis of the isoleucyl-tRNA synthetase. These results are in agreement with findings of Nass and Neidhardt (7), who made similar observations by using the same *E. coli* strain, NP2, employed in the present study. Consistent with results of Nass and Neidhardt (7), we observed an immediate cessation of synthesis of this synthetase when excess isoleucine was supplied to the derepressed culture.

The rate of synthesis of leucyl-tRNA synthetase is specifically derepressed by leucine restriction and repressed by the addition of leucine to a derepressed culture. In this respect, regulation of synthesis of leucyl-tRNA synthetase is qualitatively identical to that of isoleucyl-tRNA synthetase (i.e., the rate of synthesis is specifically affected by the supply of the cognate amino acid to the cells).

The data in this report extend the findings of Nass and Neidhardt (7) and Williams and Neidhardt (10), in which regulation of synthesis of phenylalanyl- and isoleucyl-, and arginyl-, and histidyl-tRNA synthetases, respectively, were reported. Thus, regulation of synthesis by a repression-like mechanism has now been demonstrated for 6 of the 20 aminoacyl-tRNA synthetases. A survey to ascertain whether the synthesis of other aminoacyl-tRNA synthetases is regulated is needed before regulation of synthetases is established as a general concept. Thus, we are presently examining the question of regulation of synthesis of four other aminoacyl-tRNA synthetases, especially the lysyl-tRNA synthetase whose activity has been shown to vary many fold in a lysyl-tRNA synthetase mutant of *E. coli* (I. N. Hirschfield and P. C. Zamecnik, Bacteriol. Proc., p. 136, 1970).

The question of inactivation (loss of measurable activity) of aminoacyl-tRNA synthetase

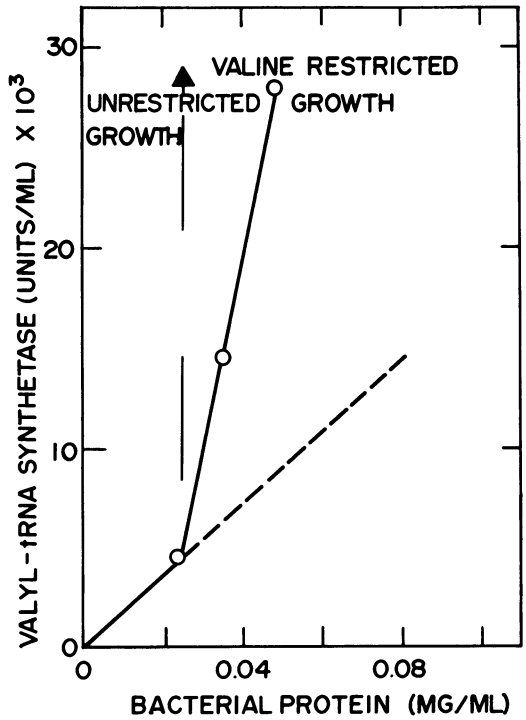

FIG. 11. *Differential de novo rate of formation of valyl-tRNA synthetase of strain M4862 H-5 as determined by density labeling. Results shown in this Fig. are calculated from the rates of synthesis measured in Fig. 9 and listed in Table 2. Data are expressed as total enzyme units per milliliter of culture taken from Table 2 as a function of total protein per milliliter of culture. Deuterium oxide unrestricted to water-valine limitation shift occurred at the time indicated by the arrow.*

remains an intriguing one. Whatever the basis of the inactivation process, it has been observed for valyl-, isoleucyl-, and leucyl-tRNA synthetases in the present study and for arginyl-, histidyl-, and isoleucyl-tRNA synthetases in studies reported by Williams and Neidhardt (10). From an experimental point of view, it is essential that one is careful to determine the extent to which inactivation of activity influences the apparent change due to different rates of synthesis. Both of the processes (derepression and inactivation) can occur under some conditions of severe limitation of a required amino acid.

The structural genes of isoleucyl-, valyl-, and leucyl-tRNA synthetases lie on the *E. coli* and *S. typhimurium* chromosomes at some distance from the genes affecting the formation of their respective amino acids (1, 2, 8, 9). It, therefore, might be expected that the two classes of enzymes would not be controlled in a coordinate manner. That they are controlled in an almost parallel manner raises the question of whether similar regulatory elements might be involved for the two classes of enzymes. To examine this possibility, we plan studies of synthetase regulation in mutants exhibiting altered regulation of the biosynthetic enzymes.

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