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Specificity of Aminoacyl Transfer Ribonucleic Acid Synthetases from *Escherichia coli* K12*

Masatoshi Kondo† and Carl R. Woese

ABSTRACT: The isoleucyl, valyl, and leucyl transfer ribonucleic acid synthetases from *Escherichia coli* K12 are isolated. Their specificity for amino acids is studied by the adenosine triphosphate–inorganic pyrophosphate exchange and aminoacyl transfer ribonucleic acid synthesis reactions. The isoleucyl transfer ribonucleic acid synthetase from strain K12 is found to recognize L-leucine and L-valine as well as L-isoleucine by the adenosine triphosphate–inorganic pyrophosphate-exchange reaction. L-Leucine (and L-valine) competitively inhibits the charging of isoleucyl transfer ribonucleic acid with L-isoleucine. Interestingly, the specificity patterns shown by the aminoacyl transfer ribonucleic acid synthetases isolated from *Escherichia coli* K12 are not in all cases the same as those manifested by *Escherichia coli* B. The isoleucyl transfer ribonucleic acid synthetase from strain K12 is able to form "erroneous enzyme–aminoacyl adenylate complexes" with both L-leucine and L-valine as well as the "correct enzyme complex" with L-isoleucine. However, neither L-leucine nor L-valine can be transferred to any transfer ribonucleic acid species from such erroneous enzyme complexes. The kinetics of [^{32}P]inorganic phosphate release from the enzyme–valyl adenylate complex induced by transfer ribonucleic acid was compared with those observed with the enzyme–isoleucyl adenylate complex.

The group of enzymes known as AA-tRNA synthetases is of special interest in that they are major factors in effecting the relationship between the amino acid and its codons. The enzymes are complex and they carry out two successive reactions; the first a recognition of the amino acid and its conversion into the enzyme-bound aminoacyl adenylate, the second a transfer of the amino acid to one of its tRNAs (Hoagland *et al.*, 1958; Berg, 1961). In the specificity or accuracy with which these reactions are accomplished lies the essence of the cell's capacity to synthesize proteins of unique primary structure.

It is likely that the rate of translation mistakes does not exceed more than a few parts in 10^4/codon per translation in most cases (Loftfield, 1963; Szer and Ochoa, 1964). On the other hand, the mistake level in the formation of the enzyme–aminoacyl adenylate complex has been estimated to be as high as 5%, on the basis of the theoretical calculations (Pauling, 1958) and experimental data (Loftfield and Eigner, 1961, 1965; Bergmann *et al.*, 1961), when the enzyme has to distinguish between very similar amino acids. In fact, Norris and Berg (1964) and Baldwin and Berg (1966) have isolated a valyl adenylate complex bound to the isoleucyl-tRNA synthetase from *Escherichia coli* B. This "erroneous enzyme–valyl adenylate complex" is unable to transfer the valyl moiety to any tRNA species whatsoever and is broken down to enzyme, free valine, and AMP upon contact with tRNA specific for L-isoleucine.

In this report, we should like to present another observation of an erroneous amino acid activation by an AA-tRNA synthetase. In *Escherichia coli* K12 we have found that not only L-isoleucine and L-valine, but also L-leucine can be activated by the isoleucyl-tRNA synthetase. However, neither L-leucine nor L-valine can be transferred to any tRNA species

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from such erroneous enzyme–aminoacyl adenylate complexes. In their interactions with the isoleucyl-tRNA synthetase from *Escherichia coli* K12, L-leucine and L-valine behave similarly in all respects.

Experimental Methods

Growth of E. coli K12. *E. coli* K12 (λ) was kindly provided by Dr. J. Drake. The cells were grown in a medium containing Difco yeast extract, 0.6%; K_2HPO_4, 1.1%; KH_2PO_4, 0.85%; and glucose, 1%. The cells were also grown in the same medium by the Grain Processing Corp. (Muscatine, Iowa).

Isolation of AA-tRNA Synthetases. The AA-tRNA synthetases were isolated by the procedure described by Bergmann *et al.* (1961) with a slight modification. *E. coli* K12 cells (50 g) were suspended in 100 ml of 0.025 M Tris-HCl buffer (pH 8.0), containing 0.01 M $MgCl_2$ and 0.01 M β-mercaptoethanol, and disrupted by a French press at about 15,000 psi. To the $(NH_4)_2SO_4$ fraction 1 (Bergmann *et al.*, 1961) were added K_2ATP (pH 7.1, 20 mM), $MgCl_2$ (20 mM), K_2HPO_4 (30 mM), KF (8.5 mM), and L-valine (20 mM) in the final concentration; and the resulting mixture was heated at 45° for 45 min. The $(NH_4)_2SO_4$ fraction 2 was chromatographed on a DEAE-cellulose column (2.1 × 30 cm) and eluted by a linear increasing gradient of potassium phosphate buffer (0.02–0.25 M) with decreasing pH (7.5–6.5), a total volume of 2000 ml. This buffer contained 0.05 M β-mercaptoethanol; 12-ml fractions were collected and a flow rate was controlled to approximately 1 ml/min. For the isoleucyl-, valyl-, and leucyl-tRNA synthetase preparations, the active fractions identified by ATP–PP_i exchange were combined and concentrated by ammonium sulfate precipitation followed by overnight dialysis against 0.01 M potassium phosphate buffer (pH 7.5), containing 0.1 mM reduced glutathione (buffer changed once). The final preparations were made 10% glycerol and stored at −18°.

Preparation of tRNA. *E. coli* K12 tRNA was prepared by a modification of the method of von Ehrenstein and Lipmann (1961). The precipitate of tRNA after phenol extraction was dissolved in 0.1 M Tris-HCl buffer (pH 8.8) and dialyzed against the same buffer overnight. The dialysate was then incubated at 37° for 2–4 hr to remove any attached amino acids. The final purified preparation was stored as a lyophilized powder at −18°.

Fractionation of tRNA on Methylated Albumin Kieselguhr (MAK)[1] *Columns.* tRNA was fractionated on a one-layer MAK column (Yamane and Sueoka, 1963) with a linear saline gradient (0.4–0.8 M NaCl) in 0.05 M sodium phosphate buffer, final pH 6.7, in a total volume of 1100 ml. Fractions (3-ml) were generally collected, on which acceptor activities for L-isoleucine and L-valine were located by the aminoacylation of tRNA. The major active fractions in any peak were combined and dialyzed against 0.1 M Tris-HCl buffer (pH 8.8) overnight.

Amino Acid Dependent ATP–PP_i-Exchange Reaction. The complete reaction mixture of 0.5 ml included the following components in the final concentration: Tris-HCl (pH 8.0, 100 mM), $MgCl_2$ (5 mM), K_2ATP (2 mM), KF (10 mM), β-mercaptoethanol (10 mM), [^{32}P]$Na_4P_2O_7$ (2 mM), L-amino acid (2 mM), and an appropriate amount of enzyme. It was generally incubated at 30° for 15 min. The amount of [^{32}P]-ATP formed was measured by acid-washed Norit method described by DeMoss and Novelli (1956).

Aminoacylation of tRNA. The reaction mixture contained in a volume of 0.55 ml the following constituents in the final concentration: Tris-HCl (pH 7.3, 100 mM), $MgCl_2$ (10 mM), KCl (10 mM), K_2ATP (2 mM), reduced glutathione (8 mM), tRNA (6–10 OD_{260} units, otherwise specified), L-[^{14}C]amino acid as indicated, and an appropriate amount of enzyme. The reaction mixture was incubated at 30° and stopped by pipetting aliquots into cold 5% trichloroacetic acid (Yamane and Sueoka, 1964). The radioactivity of [^{14}C]AA-tRNA formed was determined by counting cold trichloroacetic acid insoluble material collected on a nitrocellulose filter under toluene based scintillation fluid in a Nuclear-Chicago (three channel) scintillation spectrometer.

Isolation of Enzyme–Aminoacyl Adenylate Complexes. The enzyme–aminoacyl adenylate complexes were prepared essentially by the method of Norris and Berg (1964) with some modifications. The reaction mixture for the "correct enzyme complex" contained in the final concentration: potassium phosphate buffer (pH 7.5, 2.5 mM), $MgCl_2$ (6.4 mM), K_2ATP (0.65 mM), L-[^{14}C]isoleucine (0.14–0.38 mM), β-mercaptoethanol (13 mM), and 0.1–0.4 mg of the isoleucyl-tRNA synthetase preparation in a total volume of 0.3 ml. In certain experiments as indicated, ATP was replaced by [α-^{32}P]ATP. The reaction mixture for the erroneous enzyme complexes consisted of the same constituents except 2.5 mM of [^{14}C]- or L-[^{3}H]leucine or L-[^{14}C]valine in place of L-isoleucine. The reaction mixture was incubated at 30° for 5 min, then chilled to 0°, and introduced onto the top of an ice–water-jacketed Sephadex superfine G-25 column (0.75 × 15 cm). The column was eluted into one fraction with 2.0 ml of 0.05 M sodium succinate buffer (pH 6.0) containing 0.05 M KCl, 0.01 M β-mercaptoethanol, and 0.001 M Na_2-EDTA, and further eluted with the same buffer by collecting dropwise 0.33-ml fractions. The radioactivity of the enzyme complex formed was measured by direct counting of a portion of each fraction. The peak fractions in the enzyme complex region were pooled and used immediately in the subsequent experiments.

Reaction of Isolated Enzyme–Aminoacyl Adenylate Complexes with tRNA. An estimated 0.02–0.1 mμmole of the isolated enzyme–aminoacyl adenylate complex was added to either total or fractionated tRNA as indicated. The reaction mixture was made up to a total volume of 0.55 ml with sodium succinate buffer described above and incubated at 0°. When aminoacylation of tRNA was measured, the enzyme complex was labeled with a radioactive amino acid, and the amount of charged tRNA was determined as described. In studies where AMP release from the enzyme complex was determined, the method of Baldwin and Berg (1966) was employed, *i.e.*, [^{32}P]P_i was released from the free AMP by alkaline phosphatase and measured by charcoal adsorbable *vs.* nonadsorbable counts.

Materials

K_2ATP was purchased from P-L Laboratories. Reduced glutathione was obtained from Mann Research Laboratories.

[1] Abbreviations used are: $tRNA^x$, tRNA fraction being able to accept x; $tRNA^{x1}$, identified by superscript in order of elution on a methylated albumin kieselguhr column; MAK, methylated albumin kieselguhr.

TABLE I: Specificity of Isoleucyl-, Valyl-, and Leucyl-tRNA Synthetases from *E. coli* K12.[a]

Amino Acids Tested	Isoleucyl-tRNA Synthetase	Valyl-tRNA Synthetase	Leucyl-tRNA Synthetase
L-Isoleucine	1	0.102 (0.01)	0.06 (<0.01)
L-Valine	0.57 (0.44–0.56)	1	0.05 (0.04)
L-Leucine	0.62 (0.02–0.05)	0.0003 (<0.01)	1
L-Methionine	0.09 (0.05–0.10)	0.03 (<0.01)	0 (0.02)
L-Threonine	0.05 (<0.01)	0.04 (0.28)	0 (<0.01)

[a] Enzymatic activities were measured by the ATP–PP_i-exchange reaction as described under Experimental Methods. The exchange rate for the specific enzyme–amino acid combination is given the value of unity, and all other values are normalized accordingly. The values in parentheses are those reported for the analogous *E. coli* B enzymes under the same conditions by Bergmann *et al.* (1961).

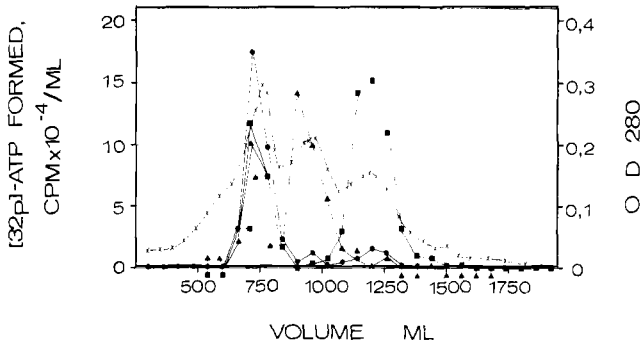

FIGURE 1: DEAE-cellulose column chromatographic separation of isoleucyl-, valyl-, and leucyl-tRNA synthetases in *E. coli* K12. The isolation and the measurement of activities of these enzymes by the ATP–PP_i-exchange reaction were carried out as described in Experimental Methods. The amounts of ATP–PP_i exchange ([^{32}P]$Na_4P_2O_7$, 2 mM; 2.5 × 10^4 cpm/μmole) for L-isoleucine (●—●), for L-valine (▲—▲), and for L-leucine (■—■) are shown and the optical density at 280 mμ is represented by x's.

Trizma base and DEAE-cellulose (medium mesh, capacity 1.0 mequiv/g) were purchased from Sigma Biochemicals. β-Mercaptoethanol was obtained from California Biochemical Corp. Alkaline phosphatase was obtained from Worthington Biochemical Corp. Isotopically labeled amino acids and [^{32}P]-$Na_4P_2O_7$ were purchased from the New England Nuclear Corp. [α-^{32}P]ATP was obtained from International Chemical and Nuclear Corp.

Results

Isolation and Specificity Patterns of Isoleucyl-, Valyl-, and Leucyl-tRNA Synthetases from E. coli K12. The chromatographic separation of the three AAtRNA synthetases by DEAE-cellulose column is shown in Figure 1. The enzymatic activities, as measured by ATP–PP_i exchange, are well separated from each other and correspond roughly to the three peaks of the optical density profile. Each AA-tRNA synthetase was collected from its peak fractions (generally about 80 ml in total) and concentrated as described under Experimental Methods, resulting in an enzyme preparation whose specific activity had been increased 50–100-fold.

Specificity patterns for each of these synthetases can be determined in either of two ways, by the ATP–PP_i-exchange rates (Table I) or by the inhibition of the aminoacylation of tRNA (Table II). In Table I the values in parentheses are those reported for the analogous synthetases isolated from *E. coli* B by Bergmann *et al.* (1961). It will be noticed that several striking differences in enzyme specificity exist between these two strains of *E. coli:* Firstly, the isoleucyl-tRNA synthetase preparation from strain K12 catalyzes ATP–PP_i exchange in the presence of L-leucine to a level that is 60% of the value obtained with L-isoleucine, but the corresponding enzyme preparation from strain B uses L-leucine to a nearly negligible extent. Secondly, L-threonine gives significant amounts of exchange with the valyl-tRNA synthetase preparation from *E. coli* B, but almost none with the corresponding enzyme preparation from *E. coli* K12. A less striking difference between two strains also appears to exist for L-isoleucine-dependent ATP–PP_i exchange with the valyl-tRNA synthetase preparations. Table II shows that in addition to giving significant ATP–PP_i exchange L-leucine and L-valine further inhibit the aminoacylation of $tRNA^{Ile}$ with L-isoleucine by the corresponding enzyme. Further, inhibition of the same reaction by L-norvaline is also significant.

On the basis of these data alone, however, it is not possible to state unequivocally that the supposed L-leucine (or L-valine) interactions with the isoleucyl-tRNA synthetase are not merely due to certain artifacts. In particular, a contamination of the isoleucyl-tRNA synthetase preparation with a

TABLE II: Specific Inhibition of the Formation of Isoleucyl-tRNA.[a]

Analogs	Inhibn on Initial Rate of Reaction (%)
L-Threonine	0
L-Methionine	0
L-α-Amino-*n*-butyric acid	0
L-Norleucine	0
L-Norvaline	*ca.* 18
L-Valine[b]	*ca.* 30
L-Leucine[c]	*ca.* 40

[a] 2.0 × 10^{-6} M of L-isoleucine, 1 × 10^{-5} M of total tRNA, and 2.0 × 10^{-4} M analogs in the final concentration were in the reaction mixture. Other experimental conditions are as described in Experimental Methods. [b] 2.91 × 10^{-7} M of L-isoleucine and 1.82 × 10^{-4} M of L-valine were used. [c] 1.82 × 10^{-4} M of L-leucine was used.

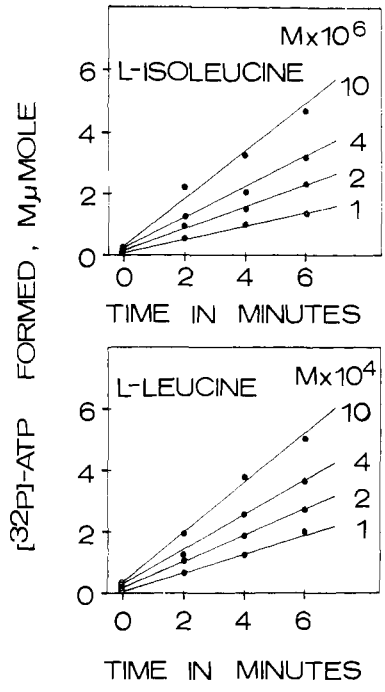

FIGURE 2: L-Isoleucine- and L-leucine-dependent ATP–PP_i-exchange reaction with the isoleucyl-tRNA synthetase preparation from *E. coli* K12. The reaction mixture of 0.5 ml contained either L-isoleucine or L-leucine at the concentration indicated on the right-hand side of the figure, and [^{32}P]$Na_4P_2O_7$ (2.75×10^5 cpm/μmole), 2 mM; ATP (dipotassium salt), 2 mM; enzyme, 2.8 μg; and other components as described in Experimental Methods. Aliquots of 0.1 ml were removed, and the radioactive [^{32}P]ATP formed was measured by acid-washed Norit method (DeMoss and Novelli, 1956).

leucyl- (or a valyl-) tRNA synthetase must be considered, as must a contamination of the L-leucine (or L-valine) with traces of L-isoleucine. With regard to these alternatives, we cite the following evidence. (1) The levels of L-leucine needed to give measurable ATP–PP_i exchange with this enzyme preparation are approximately two orders of magnitude higher than those

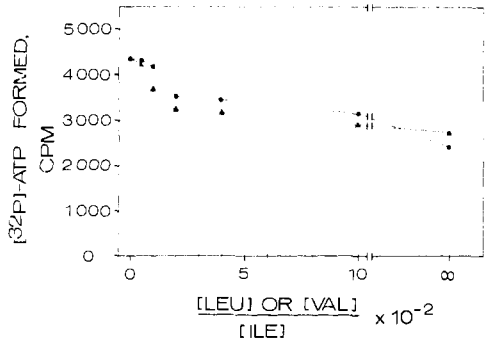

FIGURE 3: Competition between L-leucine or L-valine and L-isoleucine for the isoleucyl-tRNA synthetase as measured by the ATP–PP_i-exchange reaction. The concentration of L-isoleucine (final concentration, 2 mM) was held constant and that of either L-leucine or L-valine was varied to give the ratios shown on the abscissa. (For the ∞ value, no L-isoleucine was present.) The reaction mixture was incubated at 30° for 30 min and [^{32}P]ATP formed with [^{32}P]$Na_4P_2O_7$ (2 mM; 1.2×10^6 cpm/μmole) was measured as in Figure 2; L-isoleucine plus L-leucine (▲—▲) or plus L-valine (●—●).

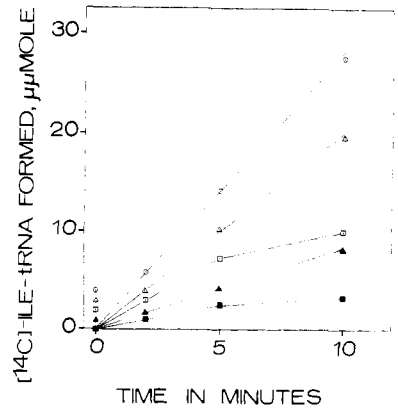

FIGURE 4: Inhibition of [^{14}C]isoleucyl-tRNA formation by L-leucine or L-valine. The reaction mixture (0.55 ml) containing L-[^{14}C]isoleucine (final concentration of 2.91×10^{-7} M; 4.6×10^5 cpm/μmole); 5.76 OD_{260} units of total tRNA; 0.25 μg of the isoleucyl-tRNA synthetase preparation, and other required components was incubated at 30° in the presence and absence of L-leucine or L-valine. Aliquots of 0.1 ml were removed at prescribed intervals and the [^{14}C]isoleucyl-tRNA formed was precipitated and washed on a nitrocellulose filter with 20 ml of cold 5% trichloroacetic acid. The dried filter was counted under 15 ml of toluene–2,5-diphenyloxazole –1,4-bis[2-(5-phenyloxazolyl)]benzene scintillation fluid. L-Isoleucine alone (⊙—⊙); plus L-valine, 1.82×10^{-4} M (△—△); or 9.10×10^{-4} M (▲—▲); and plus L-leucine, 1.82×10^{-4} M (⊡—⊡), or 9.10×10^{-4} M (■—■).

of either L-leucine or L-isoleucine needed to give comparable ATP–PP_i exchange rates with the leucyl- or the isoleucyl-tRNA synthetases, respectively; *i.e.*, the K_m of this enzyme preparation must be abnormally high (see Figures 2 and 3). The same is, of course, true for L-valine. (2) In the presence of a sufficiently high level of L-leucine or L-valine the maximum rate of L-isoleucine-directed ATP–PP_i exchange can be reduced to about 60% of its normal maximum value, *i.e.*, it is reduced to the maximum ATP–PP_i-exchange rate attainable by the K12 isoleucyl-tRNA synthetase preparation with L-leucine or L-valine alone (see Figure 3). (3) This enzyme preparation in question does not place (labeled) L-leucine or L-valine on any tRNA to a measurable extent (see Figure 6). Thus L-leucine and L-valine can inhibit the L-isoleucine-directed ATP–PP_i exchange with this enzyme preparation. High levels of L-isoleucine alone do not, of course, lower the maximum ATP–PP_i exchange rate in the present system. These facts cannot be explained by the presence of a leucyl- or a valyl-tRNA synthetase in this enzyme preparation and/or a contamination of L-leucine or L-valine with traces of L-isoleucine. Therefore, we have concluded that the activation of L-leucine or L-valine by the isoleucyl-tRNA synthetase from *E. coli* K12 is real.

Competitive Nature of Inhibition of Isoleucyl-tRNA Synthesis by L-Leucine and L-Valine. It has been shown for the *E. coli* B system that L-valine is a competitive inhibitor of the L-isoleucyl-tRNA synthesis reaction (Bergmann *et al.*, 1961) and of the formation of L-isoleucyl hydroxamate as well (Loftfield and Eigner, 1966). We have confirmed the competitive nature of the L-valine inhibition of L-isoleucyl-tRNA formation in the present system. Figures 4 and 5 show that the same is true for the inhibition of L-isoleucyl-tRNA formation brought about by L-leucine. Figure 4 presents some

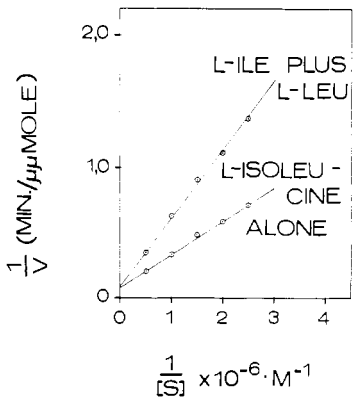

FIGURE 5: The Lineweaver–Burk plot for the formation of the isoleucyl-tRNA in the presence and absence of L-leucine. Reaction conditions are as described in Figure 4, except concentrations of L-[^{14}C]isoleucine as indicated on the abscissa and 3.4 OD_{260} units of tRNA. The concentration of L-leucine was 1.82×10^{-4} M as a final concentration when included in the reaction mixture.

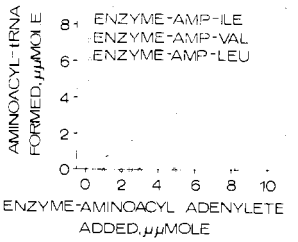

FIGURE 6: Inability of the erroneous enzyme-aminoacyl adenylate complexes to form aminoacyl-tRNA. [^{14}C]Amino acid (L-[^{14}C]valine, 2.4×10^7 cpm/μmole; L-[^{14}C]leucine, 5.6×10^7 cpm/μmole; or L-[^{14}C]isoleucine, 2.3×10^8 cpm/μmole) labeled "erroneous" or "correct" complexes of the isoleucyl-tRNA synthetase in amounts indicated on the abscissa were incubated with 0.58 OD_{260} unit of total tRNA from *E. coli* K12 at 0° for 15 min in a total volume of 0.25 ml. The amount of ^{14}C-charged tRNA formed was determined as described in Figure 4. Ordinate: micromicromole of AA-tRNA formed; abscissa: micromicromole of enzyme complex added.

data regarding the charging of $tRNA^{Ile}$ with L-[^{14}C]isoleucine in the presence and absence of different amounts of either L-leucine or L-valine, while Figure 5 is a Lineweaver–Burk plot of the L-leucine inhibition of L-isoleucyl-tRNA formation demonstrating the competitive nature of the inhibition. The K_m calculated for L-isoleucine in $tRNA^{Ile}$ charging reaction is 2.7×10^{-6} M, while the K_i for L-leucine in inhibiting this reaction is 2.6×10^{-4} M. In other experiments the inhibition constant characterizing the L-valine inhibition of $tRNA^{Ile}$ charging is found to be 4.0×10^{-4} M, a value close to that of 3.8×10^{-4} M reported in the analogous system from *E. coli* B (Bergmann *et al.*, 1961).

Further Characterization of L-*Leucine and* L-*Valine Interactions with Isoleucyl-tRNA Synthetase.* In attempting to detect erroneous enzyme–aminoacyl adenylate complex in *E. coli* K12 system, we have followed in essence the procedure of Norris and Berg (1964) (see Experimental Methods). By use of Sephadex G-25 column, we are able to isolate the erroneous enzyme complexes with both L-valine and L-leucine, separated well from unreacted ATP and free amino acid. The enzyme activity located by the ATP–PP_i-exchange reaction coincides exactly with the first (excluded) radioactive peak and the ratio of enzyme-bound AMP to bound amino acid is essentially unity. Although both erroneous enzyme complexes were formed under conditions as nearly identical as possible, approximately one-third of the erroneous enzyme–leucyl adenylate complex was detected as compared with that of L-valine case. The exact reason for this is not clear at present.

It is then of interest to test whether L-leucine so activated can be transferred to any tRNA. Figure 6 demonstrates that not only does the erroneous enzyme–valyl adenylate complex fail to produce any detectable [^{14}C]valyl-tRNA (as would be expected from the results of Norris and Berg, 1964), but the erroneous enzyme–leucyl adenylate complex fails to do this as well. Furthermore, when the tRNA-induced breakdown of such erroneous enzyme complexes is measured by the alkaline phosphatase assay, $tRNA^{Ile}$ appears to be responsible for such a breakdown of the complexes (Baldwin and Berg, 1966). Then *E. coli* K12 tRNA was fractionated by MAK column (see Experimental Methods) into one fraction rich in $tRNA^{Val}$ but lacking any $tRNA^{Ile}$ activity and a second fraction enriched in $tRNA^{Ile}$ activity. Only the second of these causes breakdown of the erroneous enzyme complexes. While this does not strictly prove $tRNA^{Ile}$ to be required for breakdown of the complexes, it is consistent with the idea, and does show

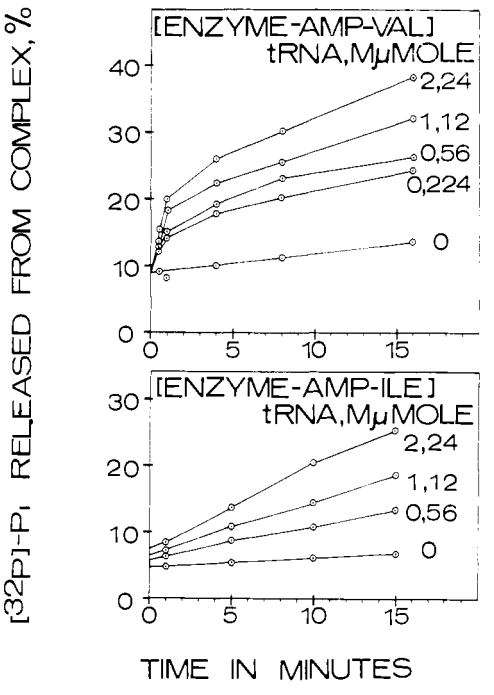

FIGURE 7: Kinetics of [^{32}P]P_i released from the erroneous enzyme–valyl adenylate *vs.* enzyme–isoleucyl adenylate complexes in the presence of tRNA. [^{32}P]AMP labeled erroneous and correct complexes of the isoleucyl-tRNA synthetase were isolated as described in the Experimental Methods, and incubated at 0° with the varying amounts of *E. coli* K12 tRNA (total) indicated on the right-hand side of the Figure. The erroneous enzyme–valyl and enzyme–isoleucyl complexes were used in the amounts of approximately 40 and 70 μμmoles, containing ^{32}P radioactivities of 3350 and 5800 cpm, respectively. AMP release from the complexes as a function of time was measured by the alkaline phosphatase assay as described by Baldwin and Berg (1966).

that $tRNA_{-}^{a}$. (and any other tRNAs in this particular fraction) will not induce such a breakdown.

Figure 7 compares the kinetics of the breakdown of the erroneous enzyme–valyl adenylate complex with those of enzyme–isoleucyl adenylate complex in the presence of varying amounts of total tRNA. The initial rate of the breakdown of such erroneous enzyme complex, measured by alkaline phosphatase release of $[^{32}P]P_i$, seems to be greater for each concentration of tRNA than that of the correct enzyme complex, at least up to 1 min. For the latter complex, the rate of reaction of AMP release is the same as that of L-isoleucyl-tRNA formation, as would be expected.

Discussion

The above results show that the specific pattern of amino acid activation by the isoleucyl- and valyl-tRNA synthetases from *E. coli* K12 differs from that characterizing the corresponding enzymes from *E. coli* B. The isoleucyl-tRNA synthetase from strain B reacts slightly if at all with L-leucine, while that from strain K12 both activates and binds L-leucine to greater extents than it does even L-valine. Similarly the valyl-tRNA synthetase from strain B readily activates L-threonine, while the corresponding enzyme from strain K12 scarcely activates this amino acid at all (Table II).

Because of the central role this class of enzymes plays in the genetic code, the differences seen in specificity spectra for the AA-tRNA synthetases are of possible use in elucidating the nature of the tRNA–tRNA synthetase interaction and certain other question bearing on the evolution of specificity in information transfer.

The results described here for *E. coli* K12 system resemble the observation in the thermophilic system in that the isoleucyl-tRNA synthetases from *Bacillus stearothermophilus* as well as *E. coli* K12 give significant amounts of ATP–PP_i exchange in the presence of not only L-isoleucine but also L-valine and L-leucine (Arca *et al.*, 1965, 1967). But they differ markedly in that at 75° L-valine (and L-leucine to a lesser extent) can be esterified to tRNA, presumably to $tRNA^{Ile}$. It must, of course, be noted in this instance that a higher temperature may distort the natural configuration of tRNA, which could possibly cause such an erroneous AA-tRNA formation. Further, in Ehrlich ascites system, the aminoacylation of tRNA seems to occur at very high concentration even with nonnatural amino acids such as L-norvaline or L-alloisoleucine (Loftfield *et al.*, 1963). It has also been observed that the presence of large amounts of leucine is strongly toxic to a mutant of *E. coli* K12 requiring phenylalanine and this toxicity can be prevented only with phenylalanine (Kindler and Ben-Gurion, 1965). It is not understood at the moment whether this *in vivo* toxicity is related to mistake making in any one of the translational steps, detected by *in vitro* experiments.

It is also interesting to note that various strains of *E. coli* in addition to showing the differences in the AA-tRNA synthetases such as reported here, appear to have differences in the "specificity" of their tRNAs. For example, strains B and K12 both produce two major classes of $tRNA^{Leu}$ separable on MAK column chromatography. However, $tRNA^{Leu1}$ from the B strain responds to poly (U,C) but not to poly (U,G) in a typical *in vitro* protein-synthesizing system (Sueoka *et al.*, 1966), whereas the analogous fraction from strain K12 responds well to poly (U,G) but to a much lesser extent poly (U,C) under these conditions (Capra and Peterkofsky, 1966).

As reported by Baldwin and Berg (1966) and we have confirmed for the present system, no more than about three-fourths of the amino acid present in the enzyme–adenylate complex ends up on tRNA. In our hands times up to 15 hr still do not yield appreciably more transfer of amino acid to tRNA from the enzyme complex than occurs after 1 hr, *i.e.*, 70% transfer. The reason for this is not clear, but it may be possible that the aminoacyl adenylate moiety dissociates in part from the enzyme upon contact with specific tRNA before being esterified to tRNA.

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