

Isolation and Partial Characterization of Temperature-Sensitive *Escherichia coli* Mutants with Altered Leucyl- and Seryl-Transfer Ribonucleic Acid Synthetases

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Two temperature-sensitive mutants of *Escherichia coli* have been found in which the conditional growth is a result of a thermosensitive leucyl-transfer ribonucleic acid (tRNA) synthetase and seryl-tRNA synthetase, respectively. The corresponding genetic loci, *leuS* and *serS*, cotransduce with *lip* and *serC*, respectively. As a result of the mutationally altered leucyl-tRNA synthetase, some leucine-, valine-, and isoleucine-forming enzymes were derepressed. Thus, leucyl-tRNA synthetase is involved in the repression of the enzymes needed for the synthesis of branched-chain amino acids.

During the last few years, mutants of *Escherichia coli* have been described in which altered aminoacyl-transfer ribonucleic acid (tRNA) synthetases for a dozen different amino acids have been found. Most of these are conditional mutants. They contain thermolabile aminoacyl-tRNA synthetases which confer thermosensitivity to the bacterium, as the particular aminoacyl-tRNA is not formed at the nonpermissive temperature (22, 28, 38). Aminoacyl-tRNA synthetases with altered affinity for their amino acids are found among amino acid auxotrophs (8, 10, 15, 17, 28, 33). In some cases, mutants of aminoacyl-tRNA synthetases have been shown to confer resistance to amino acid analogues (9, 13, 31, 36). In addition, studies of the pathways leading to amino acid biosynthesis have revealed mutants of aminoacyl-tRNA synthetases which affect the biosynthesis of the particular amino acid (31). In many instances, these mutant strains shed light on the involvement of aminoacyl-tRNA synthetases or their reaction products on the regulation of the enzymes needed for amino acid biosynthesis (3).

All the information about mutants is consistent with the idea that in *E. coli* there exists one aminoacyl-tRNA synthetase specific for each amino acid. As there frequently are multiple tRNA species for a particular amino acid, these isoacceptor tRNA molecules must be charged by the same enzyme.

We felt that it was important to obtain addi-

tional aminoacyl-tRNA synthetase mutants to study structure-function relationships with these enzymes and to ascertain that there was only one enzyme catalyzing the aminoacylation of multiple-isoacceptor tRNA species. In this report we discuss the isolation of a thermosensitive leucyl-tRNA synthetase (*LeuRS*) mutant and a thermosensitive seryl-tRNA synthetase (*SerRS*) mutant which were found when temperature-sensitive *E. coli* mutants were examined.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are described in Table 1. Figure 1 shows the genetic map of *E. coli* with the location of pertinent markers. In the text only the relevant genes are indicated after the strain number.

Media and reagents. The medium of Luria and Burrows (LB) was prepared as described previously (25). For some growth curves and for selection of transductants, minimal medium 56/2 was used (1), supplemented with any necessary growth factors, such as thiamine (0.1 $\mu\text{g/ml}$), amino acids (each, 100 $\mu\text{g/ml}$), purines and pyrimidines (each, 40 $\mu\text{g/ml}$), and glucose (0.2%) or succinate (0.4%).

Uniformly labeled ^{14}C -amino acids were obtained commercially and had the following specific activities (mCi/mole): leucine, 247; serine, 160. The other 18 natural ^{14}C -amino acids had specific activities between 50 and 470. *E. coli* B tRNA was purchased from Schwarz BioResearch, Inc., Orangeburg, N. J. Antisera against the pure *E. coli* K-12 *LeuRS* and *SerRS* were gifts of G. Myers and J. Katze, respectively.

Isolation of mutants. Temperature-sensitive mutants

TABLE 1. Description of bacterial strains^a

Strain	Genotype	Source
D ₂	F ⁻ <i>thy-35 str-120</i>	S. Kaplan (18)
KL231	F ⁻ <i>thy-35 str-120 leuS31</i>	NG Treatment of D ₂
KL232	F ⁻ <i>thy-35 str-120 leuS31R1</i>	TR revertant of KL231
KL233	F ⁻ <i>thy-35 str-120 leuS31R2</i>	TR revertant of KL231
KL234	F ⁻ <i>thy-35 str-120 leuS31R3</i>	TR revertant of KL231
AT1325lip9	F ⁻ <i>lip-9 proA2 purB15 his-4 thi-1 str-35 lacY1 galK2 mtl-1 xyl-5</i>	J. R. Guest (12)
KL229	F ⁻ <i>thy-35 str-120 serS15</i>	NG treatment of D ₂
KL282	Hfr (Cavalli) <i>phoA4 supD32 rel-1 serC13 tonA22 T2^R</i>	S. Clarke, W. Konigsberg, and B. Low, (unpublished data)
KL235	Hfr (Cavalli) <i>phoA4 supD32 rel-1 serS15 tonA22 T2^R</i>	<i>serC⁺ serS15</i> transductant of KL282
KL236	Hfr (Cavalli) <i>phoA4 supD32 rel-1 serS15R4 tonA22 T2^R</i>	TR revertant of KL235
KL237	Hfr (Cavalli) <i>phoA4 supD32 rel-1 serS15R5 tonA22 T2^R</i>	TR revertant of KL235
F ₂ -gal/W3101	F ₂ -gal/galK22	J. R. Guest (12)
X573	ORF4/ser-12 lac-lip deletion	J. R. Guest (12)

^a Genetic symbols are as described by Taylor (37). Abbreviations: leuS, locus for leucyl-tRNA synthetase; NG, nitrosoguanidine; TR, temperature-resistant.

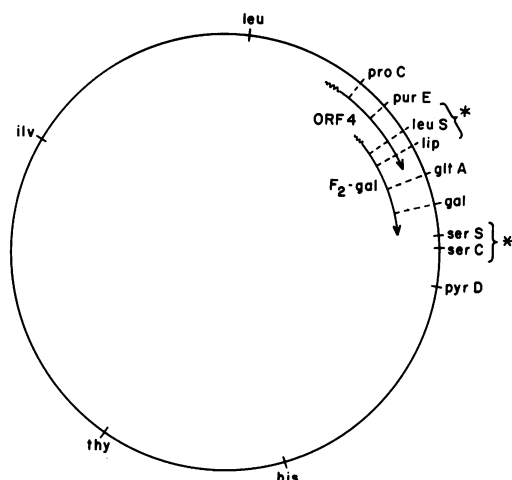


FIG. 1. Genetic map of *Escherichia coli* showing relative positions of genetic loci used in this study. Genetic symbols are those described by Taylor (37). Map positions were obtained from references 12, 14, and 37 and from the present investigation.* Relative positions were not determined.

were isolated after nitrosoguanidine mutagenesis (2) and selection by thymineless death at 42 C. The experimental conditions were exactly as described by Kaplan and Anderson (18), except that cells from exponential phase were mutagenized. The strains were tested on minimal medium supplemented by amino acids, purines, and pyrimidines at 30 and 42 C.

Bacterial crosses. The transfer of F-prime factors was carried out in LB medium. Donor cells were grown at 37 C and recipients at 30 C to a concentration of 2

$\times 10^8$ cells/ml. Cells were mixed in a 1:10 donor-recipient ratio and gently aerated for 1 hr at 37 C. They were then plated onto LB agar plates which contained 100 μ g of streptomycin per ml to kill the donor cells. The plates were incubated at 42 C to select for temperature-resistant recombinants.

Transductions were carried out by using P1 vir (a gift of J. Tomizawa). Lysates of temperature-sensitive donor strains were prepared by adsorbing 10^7 P1 vir plaque-forming units onto 5×10^7 freshly grown cells (5×10^8 /ml) in LB medium which contained 2.5 mM Ca²⁺. After 20 min of adsorption at 37 C, 4 ml of 0.4% agar was added, and the mixture was poured onto a Z plate (LB medium, 1% agar, 0.1% glucose, and 2.5 mM Ca²⁺). After overnight incubation at 30 C, the top agar was scraped into a centrifuge tube and mashed for 5 min with several drops of chloroform. After centrifugation, the supernatant fluid was saved as the lysate. Transductions were begun by adsorbing phage and bacteria in a 1:5 ratio in LB medium containing 2.5 mM Ca²⁺ as described above. The cells were then washed twice by centrifugation and plated onto recombinant-selective plates.

Growth of bacteria. Usually bacteria were grown in flasks on a rotary shaker at 30 C in LB medium containing 20 μ g of thymidine per ml. Cultures for cell extracts were grown to stationary phase. For the experiments with the biosynthetic enzymes for branched-chain amino acids, cells were grown in the same medium at 39 C and chilled when they reached late exponential phase. The cells were collected by centrifugation, washed with a solution of 0.5% NaCl and 0.5% KCl, and quickly frozen.

Preparation of cell extracts. Frozen cells were opened by grinding with alumina. After addition of standard buffer [0.01 M tris(hydroxymethyl) aminomethane-hydrochloride (pH 7.5), 0.01 M MgCl₂, 0.02 M β -

mercaptoethanol, 0.01 M NH_4Cl , and 10% (v/v) glycerol], the mixture was centrifuged at $10,000 \times g$ to remove alumina and cell debris. Ribosomes were removed by centrifugation for 2 hr at $198,000 \times g$. The resulting supernatant fluid was dialyzed against standard buffer, glycerol was added to a final concentration of 50%, and the mixture was stored at -20°C . The extracts at this stage normally contained between 3 and 9 mg of protein per ml, as determined by the method of Lowry et al. (24), with crystalline bovine plasma albumin as standard.

Enzyme assays. Aminoacyl-tRNA synthetases were assayed at 30 and 42 C by the aminoacylation reaction (32) or the adenosine triphosphate-inorganic polyphosphate (ATP-PP_i) exchange (6) reaction. The aminoacylation reaction mixture contained (per ml): sodium cacodylate (pH 7.2), 100 μmoles ; MgCl_2 , 10 μmoles ; KCl, 10 μmoles ; ATP, 2 μmoles ; ^{14}C -amino acid, 2 nmole; crude tRNA, 20 A_{260} units; bovine plasma albumin, 0.1 mg; and diluted cell extract in a solution containing 10 mM sodium cacodylate, 10 mM 2-mercaptoethanol, 1 mg of bovine plasma albumin per ml, and 10% (v/v) glycerol. After incubation for 2 or 3 min (in the linear region to give initial velocity), portions were used for assay of acid-precipitable radioactivity by the filter-paper technique. One unit of enzyme activity is defined as the amount forming 1 nmole of aminoacyl-tRNA in 2 min under the conditions described.

ATP-PP_i exchange was performed as described by Calendar and Berg (6). The concentration of PP_i was different in the assays for LeuRS and SerRS, as the latter enzyme is strongly inhibited by PP_i (19). The reaction mixture contained (per ml): sodium cacodylate (pH 7.2), 100 μmoles ; MgCl_2 , 8 μmoles ; L-serine or leucine, 0.5 μmole ; sodium- ^{32}P -pyrophosphate (10^4 to 10^5 counts per min per μmole), 20 pmoles (for serine) or 1 μmole (for leucine); NaF, 20 μmoles ; ATP, 2 μmoles ; and appropriately diluted cell extract in a solution containing 10 mM sodium cacodylate, 10 mM 2-mercaptoethanol, 1 mg of bovine plasma albumin per ml, and 10% (v/v) glycerol. In all experiments, initial velocities were measured. One unit of enzyme activity is defined as that amount incorporating 1 μmole of ^{32}P -PP_i into ATP in 10 min under the conditions described.

Enzymes involved in the biosynthesis of leucine, isoleucine, valine, and histidine were assayed by the following procedures: α -isopropyl-malate synthetase (20), α -acetohydroxyacid synthetase (34), and histidinol phosphate phosphatase (4).

Heat inactivation studies of SerRS and LeuRS. Cell extracts were diluted in a solution containing 10 mM sodium cacodylate, 10 mM 2-mercaptoethanol, 1 mg of bovine plasma albumin per ml, and 10% (v/v) glycerol and were incubated. Portions were removed at certain times and assayed for residual activity at a lower temperature. SerRS was incubated at 45 C and assayed at 30 C for 2 min; LeuRS was incubated at 30 C and assayed at 20 C for 5 min.

Antiserum titration of SerRS or LeuRS. Cell extracts were diluted to about 0.5 to 1 aminoacylation units of enzyme activity per ml in a buffer consisting of

10 mM sodium cacodylate, 10 mM 2-mercaptoethanol, 1 mg of bovine plasma albumin per ml, and 10% (v/v) glycerol and then were mixed with portions of appropriately diluted antiserum (in the same buffer). After 30 min at 20 C (for SerRS) or 10 min at 0 C (for LeuRS), the remaining enzyme activity in the samples was measured by the aminoacylation assay. No correction was made for enzyme inactivation during the incubation period.

RESULTS

Isolation and initial characterization of mutants. Recently a method for enrichment of thermosensitive aminoacyl-tRNA synthetase mutants was described by Kaplan and Anderson (18). In the present study, the same method was used to select temperature-sensitive mutants of strain D₂ with the use of nitrosoguanidine as a mutagen. Sixteen mutants unable to grow at 42 C were collected. They were then tested for the activity of all aminoacyl-tRNA synthetases. These experiments revealed that seven mutants had altered valyl-tRNA synthetase. In addition, one mutant with a very reduced level of LeuRS activity and another with much reduced SerRS activity were found. The nature of the lesion leading to temperature sensitivity in the seven other strains is not known. The specific activity of all aminoacyl-tRNA synthetases in the wild-type and mutant strains is shown in Table 2. The LeuRS activity in KL231 was less than 10% of the wild-type activity when assayed at either 30 or 42 C. A similar behavior was seen for SerRS in the mutant strain KL229. The activities of all other aminoacyl-tRNA synthetases were not appreciably changed. Mixing experiments using wild-type extracts and mutant extracts showed that the reduced activity in the mutant strains was not attributable to the presence of a specific diffusible inhibitor. In addition, the extracts had been dialyzed extensively before the assays. The strains with altered valyl-tRNA synthetase activity were not further characterized.

Mapping of temperature-sensitive characters. From initial mating experiments with several Hfr strains it became clear that the temperature-sensitive loci in strains KL231 and KL229 were located between *proC* and *pyrD* on the *E. coli* map. The mutant loci were further localized by using the mutants as recipients in matings with F-prime donors (*F₂-gal* and ORF4).

When KL231 was used as a recipient, both *F₂-gal* and ORF4 donors produced very high numbers of temperature-resistant, streptomycin-resistant recombinants (10 to 20% per donor cell). This implies that the mutation in KL231 lies near the *lip* locus, in the region shared by ORF4

TABLE 2. Activity of aminoacyl-tRNA synthetases in mutant and wild-type strains in the aminoacylation reaction^a

Aminoacyl-tRNA synthetase	D ₂		KL231		KL229	
	30 C°	42 C	30 C	42 C	30 C	42 C
Alanine	0.6	0.8	0.5	0.5	0.4	0.5
Arginine	2.4	3.5	1.9	2.4	0.9	2.1
Asparagine	0.3	0.4	0.4	0.2	0.4	0.5
Aspartic acid	2.6	3.3	1.6	2.1	2.1	3.1
Cysteine	0.1	0.1	0.1	0.1	0.1	0.2
Glutamine	0.2	0.3	0.2	0.3	0.3	0.4
Glutamic acid	0.7	0.7	0.4	0.5	0.4	0.5
Glycine	1.6	2.1	2.7	2.8	1.6	2.1
Histidine	0.3	0.5	0.4	0.6	0.3	0.2
Isoleucine	1.3	1.9	0.6	1.0	1.1	1.4
Leucine	1.3	1.2	0.04	0.02	0.8	1.2
Lysine	1.8	4.5	3.5	4.0	4.0	4.6
Methionine	1.0	1.2	0.8	1.2	0.5	0.7
Phenylalanine	0.3	0.4	0.4	0.5	0.6	0.8
Proline	0.4	0.2	0.3	0.3	0.1	0.1
Serine	2.1	3.3	1.7	2.1	0.01	0.01
Threonine	0.5	0.5	0.3	0.5	0.2	0.6
Tryptophan	0.8	1.6	0.3	0.6	0.9	1.7
Tyrosine	1.5	1.4	0.9	0.9	1.1	2.1
Valine	1.5	2.5	1.4	3.2	2.6	3.7

^a Values expressed as units of activity per milligram of protein.

^b Assay temperature.

and *F₂-gal* (Fig. 1). Transduction from KL231 into AT1325*lip9* produced *lip*⁺ transductants, of which 42% were as temperature-sensitive as KL231 and 58% were temperature-resistant. Extracts from a few of these temperature-sensitive transductants were tested and found to have the very low LeuRS activity characteristic of KL231, whereas the AT1325*lip9* parent had normal LeuRS activity. We conclude that the mutation conferring low LeuRS activity, denoted *leuS31*, lies near the *lip* locus and is cotransduced with it at a frequency of 42%.

The temperature-sensitive locus in KL229 was localized by cotransduction with *serC*. *SerC* is the locus for 3-phosphoserine-transaminase, the second enzyme in the serine biosynthetic pathway (7, 29). A certain mutant described by Hoffman et al. (14) was found to require serine and was originally believed to be a SerRS mutant. Subsequent investigation has shown that the strain described by Hoffman et al. is actually a *serS*⁻ *serC*⁻ double mutant and that *serS14* and *serC* are cotransduced at a frequency of approximately 69% (S. Clarke, W. Konigsberg, and B. Low, unpublished data). When strain KL229 was used as a donor of *serC*⁺ in transductions of KL282, 64% of the transductants were found to

be temperature-sensitive. Extracts of several temperature-sensitive transductants were found to have the low SerRS activity characteristic of KL229. We conclude that the mutation causing temperature sensitivity in KL229 (denoted *serS15*) is cotransduced with *serC* at a frequency of 64%, which is very close to the value of 69% found for *serS14* (S. Clarke, W. Konigsberg, and B. Low, unpublished data).

Characterization of altered LeuRS activity. The LeuRS activity in mutant strain KL231 was tested in a crude cell extract and in a partially purified preparation free from RNA and deoxyribonucleic acid obtained by diethylaminoethyl cellulose chromatography. In both cases, a low specific activity of the enzyme was found. To find an explanation for this fact, the susceptibility of mutant and wild-type enzyme to heat inactivation was studied. The thermal denaturation of the mutant LeuRS was found to be much more rapid than that of the wild-type enzyme (Fig. 2). The mutation had a different effect on each of the two activities of LeuRS tested. The activity in the aminoacylation reaction was affected much more than was the activity in the ATP-PP_i exchange reaction (Table 3). This may result partially from the known activation of leucine by isoleucyl-tRNA synthetase (21).

It was not known whether the mutation in KL231 was regulatory mutation affecting the amount of LeuRS synthesized or rather a mutation in the structural gene for the enzyme. The results of the heat-inactivation experiments suggest the latter. This was further strengthened by a titration experiment in which the LeuRS activity was neutralized by antiserum prepared against the pure *E. coli* enzyme from *E. coli* K-12. The data in Fig. 3 show the effect of increasing amounts of antiserum on a standard amount of LeuRS activity from wild-type and mutant strains. A much larger amount of antiserum was needed to inactivate the mutant LeuRS than the wild-type LeuRS. Thus, the *leuS* mutation appears to be in the structural gene for LeuRS leading to the production of normal amounts of a thermolabile enzyme with much reduced activity.

Also, it was not known whether the mutant LeuRS could aminoacylate all the different tRNA^{Leu} isoacceptors. When large amounts of enzyme were used, five different pure *E. coli* K-12 tRNA^{Leu} species (5) could be charged fully. However, small kinetic differences in this reaction between the isoacceptors would not have been detected. The low purity of the mutant enzyme did not justify extensive *K_m* studies.

Characterization of altered SerRS activity. In

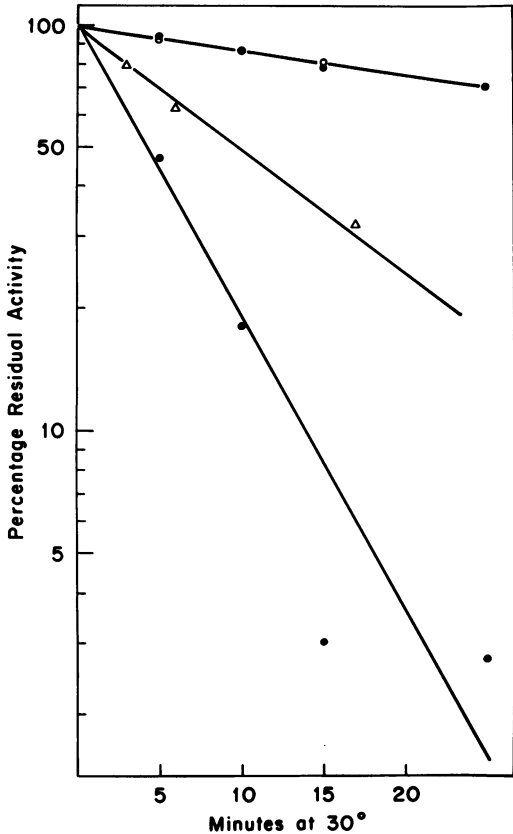


FIG. 2. Heat inactivation of *LeuRS*. Symbols: *D*₂, parent (○); KL231, mutant (●); KL232, revertant (⊙); KL234, revertant (Δ).

TABLE 3. *Leucyl-tRNA synthetase* activity in various strains^a

Reaction	Strain				
	<i>D</i> ₂	KL231	KL232	KL233	KL234
Aminoacylation . . .	1.23	0.08	2.10	2.20	0.12
ATP-PP _i exchange	2.36	0.93	2.00	2.53	0.61

^a Assayed at 30 C. Values expressed as units of activity per milligram of protein.

studies analogous to the ones described above for *LeuRS*, the low *SerRS* activity persisted when the extracts of KL229 were further purified. Heat-inactivation studies (Fig. 4) showed the mutant *SerRS* to be much more sensitive to elevated temperature. As with *LeuRS*, the mutation in KL235 had different effects on the two activities of *SerRS*: the ATP-PP_i exchange was much less affected than was the aminoacylation (Table 4). As described above, an antiserum titration

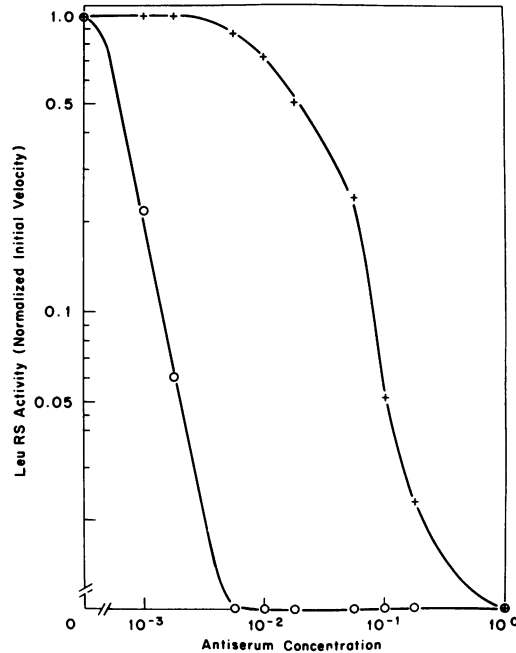


FIG. 3. Antiserum titration of *LeuRS*. Symbols: *D*₂, parent (○); KL231, mutant (+).

(Fig. 5) was performed with KL282 and KL235 to determine whether the synthesis or activity of *SerRS* was affected by the mutation. Again, for the neutralization of a fixed amount of *SerRS* activity, much more antiserum was needed with the mutant enzyme than with the wild-type enzyme. As in the case of the *LeuRS* mutant described above, this indicates that the *SerRS* activity, not the amount of *SerRS* in the cell, is altered. The ability of the mutant *SerRS* in KL235 to aminoacylate various *tRNA*^{Ser} species was also tested. Large amounts of the mutant enzyme charged five different serine isoacceptors of *E. coli* K-12 (32) to their full extent.

Reversion of mutant phenotype and comparison of growth characteristics. The two mutant strains described, KL231 and KL229, were isolated on the basis of their failure to grow at 42 C on minimal medium. This phenotype was found to be associated with the *leuS31* and *serS15* mutations as described above. It was subsequently found that growth of the mutant strains on LB medium took place at temperatures not possible on minimal medium. For example, in Table 5, which shows approximate doubling times in liquid media obtained for mutant KL231 and the parent strain *D*₂, it is seen that the mutant grew slowly in LB at 42 C but not in minimal medium. The addition of leucine (100 μg/ml) to the

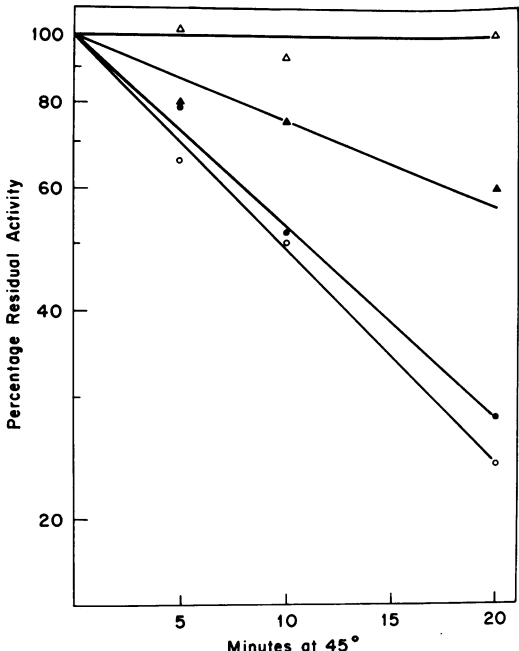


FIG. 4. Heat inactivation of SerRS. Symbols: KL282, parent (Δ); KL235, mutant (○); KL236, revertant (●); KL237, revertant (▲).

TABLE 4. Seryl-tRNA synthetase activity in various strains^a

Reaction	Strain			
	KL282	KL235	KL236	KL237
Aminoacylation	2.4	0.07	1.7	0.13
ATP-PP _i exchange	0.05	0.02	0.07	0.07

^a Assayed at 30 C. Values expressed as units of activity per milligram of protein.

minimal medium increased the growth rate at 37 C.

Revertants of the mutant strains were obtained by plating out samples of overnight cultures onto minimal agar and incubating for 2 days at 42 C. Revertants were found at a frequency of 1 colony per 10⁸ cells plated.

Some biochemical properties of the aminoacyl-tRNA synthetases in a few revertant strains are summarized in Tables 2 to 4 and in Fig. 2 and 4. The SerRS and LeuRS in revertants of KL235 and KL231, respectively, had specific activities that varied between those of wild-type strain and original thermosensitive mutant. Although all the revertant strains grew at 42 C, their LeuRS or SerRS enzymes still showed some thermostability.

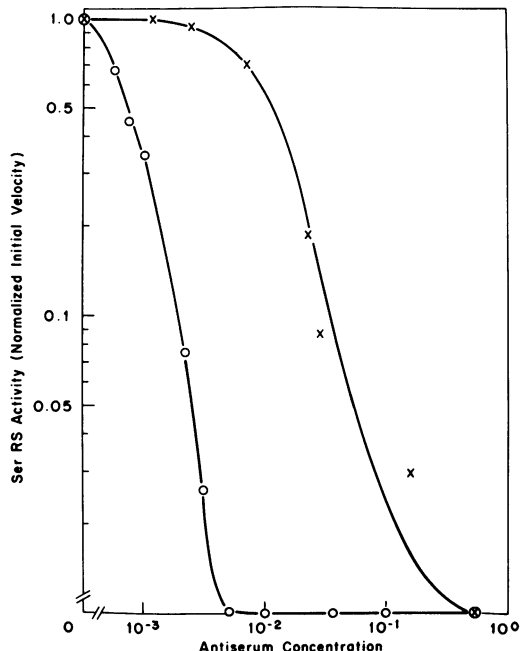


FIG. 5. Antiserum titration of SerRS. Symbols: KL282, parent (○); KL235, mutant (×).

To test the possibility that the mutant LeuRS enzyme has an altered amino acid recognition site, the KL231 mutant was tested for growth in the presence of several analogues of leucine. Trifluoroleucine and norvaline inhibited growth of KL231 and the D₂ parent at approximately the same concentration. Azaleucine, however, was found to inhibit the mutant strain much less than the parent. When the strains were grown on a minimal agar master plate and replica-plated onto minimal plates containing various amounts of azaleucine, the KL231 mutant grew in the presence of more than 5,000 μg of azaleucine per ml; D₂ was sensitive to about 100 μg/ml. In addition, in each of six *lip*⁻ transductants obtained from the *leuS* mapping experiment, azaleucine resistance was correlated with temperature-sensitive growth. This resistance was found only when the mutant was grown on minimal medium; when tested on LB plates, 100 μg of azaleucine per ml also inhibited growth of KL231.

LeuRS involvement in regulation of enzymes responsible for isoleucine-valine-leucine biosynthesis. If LeuRS or its reaction product, leucyl-tRNA, are involved in the regulation of the biosynthetic enzymes for branched-chain amino acids, then depressed levels of those enzymes would be expected when KL231, possessing a thermosensitive LeuRS, is grown at elevated temperature.

TABLE 5. *Doubling times of various strains*^a

Strain	LB medium		Minimal medium		
	42 C ^b	37 C	42 C	37 C	37 C + leucine
D ₂	30 min	31 min	80 min	60 min	Not tested
KL231	240 min	123 min	no growth	500 min	65 min
KL232	31 min	31 min	81 min	60 min	Not tested
KL233	31 min	31 min	81 min	67 min	Not tested

^a Accuracy of doubling times estimated to be $\pm 10\%$.^b Assay temperature.

Strains D₂ (wild-type LeuRS), KL231 (mutant LeuRS), and KL232 (revertant LeuRS) were grown in LB medium at 39 C. KL231 grew very slowly (doubling time, 162 min), whereas the wild-type and revertant strains grew at a normal rate (generation time, 31 min). Cells of the three strains grown at 39 C to the same cell density (late exponential phase) were harvested. The cell extracts were assayed for the activities of α -acetohydroxyacid synthetase, an enzyme involved in isoleucine-valine biosynthesis, and of α -isopropylmalate synthetase, which is involved in leucine biosynthesis. The activities of both enzymes are much higher in the mutant strain (KL231) than in the wild type or revertant (Table 6). As a control, an enzyme needed for the biosynthesis of a completely unrelated amino acid, histidine, was measured. The enzyme histidinol phosphate phosphatase had the same specific activity in these three strains. This indicates that the differences in the activities of α -acetohydroxyacid synthetase and α -isopropylmalate synthetase are not caused by the slow growth rate of the mutant. These results show that LeuRS is involved in the repression of the enzymes necessary for the synthesis of branched-chain amino acids.

DISCUSSION

The results described in this report again show that the method devised by Kaplan and Anderson (18) to enrich for thermosensitive aminoacyl-tRNA synthetase mutants is successful. Of 16 temperature-sensitive *E. coli* strains tested, 9 possessed altered aminoacyl-tRNA synthetase activities. It is curious that this method gives rise to a large number of thermosensitive valyl-tRNA synthetase mutants (18, 28, 38), whereas for many other aminoacyl-tRNA synthetases no temperature-sensitive mutants are known.

As has been noticed with all thermosensitive aminoacyl-tRNA synthetase mutants to date (28, 38), there is a discrepancy between the in vivo stability and the observed in vitro activity of the enzymes. Even though cell extracts are processed as carefully as possible, little LeuRS ac-

TABLE 6. *Levels of some enzymes involved in leucine, isoleucine, valine, and histidine biosynthesis*

Strain	LeuRS	Specific activity ^a		
		α -Acetohydroxyacid synthetase	α -Isopropylmalate synthetase	Histidinol phosphate phosphatase
D ₂	Wild type	1.3	0.58	6.3
KL231	Mutant	100.2	4.8	6.1
KL232	Revertant	1.7	0.60	5.9

^a Values expressed as micromoles of product formed per hour per milligram of protein.

tivity is observed when the enzyme is assayed at a temperature (e.g., 30 C) at which the mutant strains grow well. One possible explanation of this phenomenon is that in vivo stabilization of the mutant enzymes occurs through complex formation with substrates (tRNA, amino acid, or ATP). Because tRNA forms a tight complex with its cognate aminoacyl-tRNA synthetase, it is probable that these enzymes are bound to tRNA in the cell (26). Some mutant aminoacyl-tRNA synthetases can be protected against in vitro inactivation by the addition of amino acid or ATP to the buffers used for processing the extracts (38). Specifically, evidence for the in vitro stabilization of *E. coli* LeuRS by ATP and leucine has been obtained (G. Myers, *unpublished data*). Although we did not investigate the stabilization of the mutants LeuRS and SerRS by their substrates in vitro, the growth behavior of the *leuS* mutant in various media suggests such protection may occur in vivo. The addition of leucine to the minimal medium results in a much accelerated growth rate (Table 5).

The antiserum titration experiments confirmed the conclusions from the heat-inactivation studies. The mutations in KL231 and KL235 are in the structural genes of LeuRS and SerRS, respectively, and not in regulatory loci controlling enzyme synthesis. Mutants of the latter type were found recently for *E. coli* SerRS (S.

Clarke, W. Konigsberg, and B. Low, *unpublished data*).

Both SerRS and LeuRS are still able to charge all the major isolated isoacceptor tRNA species. Small differences in interaction of the mutant enzyme with the various isoacceptors would have gone undetected, but there seems to be no discrimination against a particular tRNA species by the mutant enzyme. The finding of the LeuRS and SerRS mutants provides genetic evidence for the existence of a single LeuRS and SerRS in *E. coli*, as suggested by biochemical studies (16, 27, 35).

A number of mutant strains containing altered aminoacyl-tRNA synthetases are resistant to analogues of the particular amino acid (28, 31, 36). In each of these cases a change in the affinity of the enzyme for the amino acid has been found. As the *leuS* mutant (KL231) is resistant to 50-fold higher concentrations of the leucine analogue azaleucine than is the parent strain, it seems probable that the mutant LeuRS has a lower K_m for this amino acid. Further biochemical characterization of the enzyme is necessary to substantiate this suggestion. This tolerance to high levels of azaleucine is critically dependent on the growth medium used before and after addition of the analogue. The higher resistance is observed only when the cells are maintained on minimal medium throughout. The possible relation of this effect to the phenomenon of adaptation of *E. coli* growth on trifluoroleucine (30; G. Myers, *unpublished data*) is not known.

Mutants of some aminoacyl-tRNA synthetases have been instrumental in demonstrating the involvement of these macromolecules in the repression of the biosynthetic enzymes for the respective amino acids. The aminoacyl-tRNA synthetase is necessary to form an active repressor, as has been elucidated for isoleucyl-tRNA synthetase in *E. coli* (15), histidyl-tRNA synthetase in *Salmonella typhimurium* (31) and *E. coli* (Nass and Neidhardt, *Bacteriol. Proc.*, p. 87, 1966), LeuRS in *S. typhimurium* (31), valyl-tRNA synthetase in *E. coli* (28), and tryptophanyl-tRNA synthetase in *E. coli* (17). In contrast, in a SerRS mutant of *E. coli* no effect on the level of a serine biosynthetic enzyme has been found (S. Clarke, W. Konigsberg, and B. Low, *unpublished data*). LeuRS in *E. coli* is involved in the repression of the enzymes required for the synthesis of isoleucine, valine, and leucine, as an alteration in LeuRS greatly derepresses the two biosynthetic enzymes tested. Based on previous work and on the results of this study, it is now established that, in addition to leucine, isoleucine, and valine (as well as pantothenic acid),

their cognate aminoacyl-tRNA synthetases are needed for complete repression of the biosynthetic enzymes for branched-chain amino acids.

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