CP1 Domain in *Escherichia coli* Leucyl-tRNA Synthetase Is Crucial for Its Editing Function†

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ABSTRACT: The amino acid discrimination by aminoacyl-tRNA synthetase is achieved through two sifting steps; amino acids larger than the cognate substrate are rejected by a "coarse sieve", while the reaction products of amino acids smaller than the cognate substrate will go through a "fine sieve" and be hydrolyzed. This "double-sieve" mechanism has been proposed for IleRS, a class I aminoacyl-tRNA synthetase. In this study, we created LeuRS-B, a mutant leucyl-tRNA synthetase from *Escherichia coli* with a duplication of the peptide fragment from Met328 to Pro368 (within its CP1 domain). This mutant has 50% of the leucylation activity of the wild-type enzyme and has the same ability to discriminate noncognate amino acids in the first step of the reaction. However, LeuRS-B can catalyze mischarging of $tRNA^{Leu}$ by methionine or isoleucine, suggesting that it is impaired in the ability to edit incorrect products. Wild-type leucyl-tRNA synthetase can edit the mischarged $tRNA^{Leu}$ made by LeuRS-B, while a separated CP1 domain cannot. These data suggest that the CP1 domain of leucyl-tRNA synthetase is crucial to the second editing sieve and that CP1 needs the structural context in leucyl-tRNA synthetase to fulfill its editing function.

Aminoacyl-tRNA synthetases (aaRSs)[1] arose early in evolution and are believed to be a group of ancient enzymes that catalyze the precise charging of tRNAs with their cognate amino acids (*1*). The aminoacylation of tRNA is a two-step reaction: (a) activation of amino acids with ATP by forming aminoacyl adenylates and (b) transferring of the aminoacyl residue from the aminoacyl adenylate to the cognate tRNA substrate (*2*). The accuracy of aminoacylation depends on both the specific recognition of amino acids during their activations (coarse sieve) and the pre- or post-transferring editing (fine sieve) that correct errors at either the aminoacyl adenylate level or the tRNA level (*3*−*5*). These editing reactions during the aminoacylation of tRNAs by aaRSs are essential for the accurate incorporation of amino acids during protein biosynthesis (*4*, *6*−*8*). Leucyl-tRNA synthetase (EC 6.1.1.4) from *Escherichia coli* is a monomeric enzyme consisting of 860 amino acid residues with a putative molecular mass of 97.3 kDa (from the *leuS* gene sequence; *9*). While LeuRS does misactivate methionine and isoleucine (*10*), in the presence of $tRNA^{Leu}$, either the misactivated amino acids or the mischarged $tRNA^{Leu}$ is hydrolyzed (*11*).

On the basis of their conserved amino acid sequences and crystal structures, aaRSs are divided into two major classes, class I and II, each with characteristic sequences and structural motifs that form the substrate binding sites and catalytic sites (*12*−*15*). The 10 class I enzymes share HIGH and KMSKS motifs and active sites based on the Rossmann fold (an overall $\beta_6\alpha_4$ structure) (*16*−*18*). The Rossmann fold is made up of two $\beta_3\alpha_2$ halves, linked by the connective polypeptide 1 (*19*, *20*). Among the 10 class I aaRSs, LeuRS, ValRS, IleRS, MetRS, and CysRS belong to one subgroup (*20*). MetRS and CysRS have relatively small CP1 domains (100 and 50 amino acids, respectively), while the other three (LeuRS, ValRS, and IleRS) have larger CP1 domains ranging from about 250 to 275 amino acids (*21*). On the basis of sequence alignments of the aaRSs in the same subgroup, CP1 of LeuRS extends from residue 126 to 389 (*22*). There is some evidence showing that the CP1 domains cloned from *Bacillus stearothermophilus* ValRS and *E. coli* IleRS have the editing function of deacylating Thr-$tRNA^{Val}$ and Val-$tRNA^{Ile}$, respectively (*23*). Further information about editing has been given by the crystal structures of *Thermus thermophilus* IleRS complexed with L-isoleucine and L-valine (*24*), but the mechanism for the editing function of LeuRSs remains elusive.

To study the function of CP1 in LeuRS, seven deletion mutants and eight insertion mutants were obtained by PCR in our laboratory (*25*). Although all the deletion mutants have no aminoacylation activity, the aminoacylation activities of four insertion mutants can be determined in vitro (*25*). Among the four insertion mutants, only two mutants, LeuRS-A and LeuRS-B, were stable enough to be purified. Upon LeuRS-A, which has a duplication of the 40 amino

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[1] Abbreviations: aaRSs, aminoacyl-tRNA synthetases; ATP, adenosine triphosphate; LeuRS, leucyl-tRNA synthetase; ValRS, valyl-tRNA synthetase; IleRS, isoleucyl-tRNA synthetase; MetRS, methionyl-tRNA synthetase; CysRS, cysteinyl-tRNA synthetase; CP1, connective polypeptide 1.

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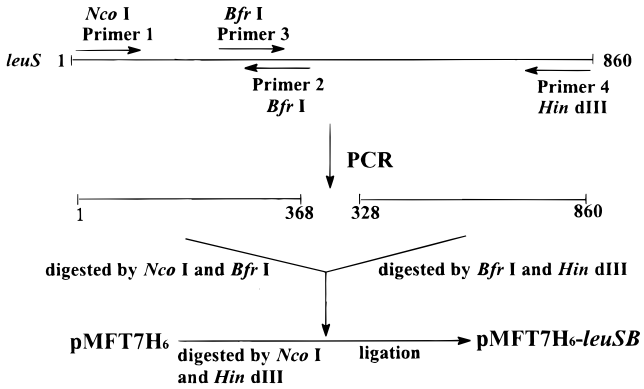

FIGURE 1: Construction of plasmid containing the gene encoding His_6-tagged LeuRS-B.

acid residues from F253 to E292, was conferred a new ability to discriminate between two isoacceptors of $tRNA^{Leu}$ (*25*). LeuRS-B, with a duplication of the peptide fragment from residue Met328 to Pro368 (in the CP1 domain), was chosen for the study of editing function, since the leuycylation activity of LeuRS-A was too low, let alone the determination of the misaminoacylation of $tRNA^{Leu}$ by it.

In this report, LeuRS-B and a stand-alone CP1 domain separated from LeuRS, named as $CP1_{Leu}$, were used to investigate the editing function of *E. coli* LeuRS. In the presence of a noncognate amino acid (methionine or isoleucine), the activity and the kinetic parameters of the two-step reactions catalyzed by LeuRS-B and LeuRS were determined. The editing activity of a purified $CP1_{Leu}$ was also studied. Here we report that the CP1 domain in *E. coli* LeuRS is essential to its editing function as a "fine sieve", and this domain needs to be in the context of *E. coli* LeuRS to fulfill this editing function. The editing mechanism of CP1 in *E. coli* LeuRS might differ from that of ValRS from *B. stearothermophilus* and IleRS from *E. coli*, in which the stand-alone CP1 domains, as independent peptides, can correct errors of aminoacylation (*23*).

EXPERIMENTAL PROCEDURES

Construction of a Plasmid Containing the Gene Encoding His_6-Tagged LeuRS-B. The gene encoding *E. coli* LeuRS, *leuS*, was cloned in our laboratory (*26*). The recombinant plasmid containing the gene encoding His_6-tagged LeuRS-B was constructed as shown in Figure 1. Two DNA fragments were obtained by PCR with *leuS* as the template; one fragment that encodes residues M1−P368 was flanked by *Nco* I and *Bfr* I restriction sites, while the other one that encodes residues M328−G860 was flanked by *Bfr*I and *Hin*dIII sites. These two DNA fragments were ligated at the *Bfr*I site, and thus formed a gene encoding the LeuRS-B mutant enzyme that produced a 41-residue duplication from M328 to P368 within the CP1 domain (*25*). The gene was then inserted into a His_6-tagging vector $pMFT7H_6$ (*27*) between *Nco*I and *Hin*dIII sites to express the N-terminally His-tagged LeuRS-B. Two amino acid residues, M328 and P368, were substituted with lysine and arginine, respectively, to introduce those restriction sites; however, these changes did not significantly affect the activity of LeuRS (unpublished data). The whole gene sequence encoding LeuRS-B was confirmed by DNA sequencing (data not shown).

Cloning and Expression of the Gene Encoding $CP1_{Leu}$. The DNA fragment encoding CP1 of LeuRS (from Thr129 to Gly389) and flanked by *Nco*I and *Hin*dIII restriction sites was generated by PCR and inserted into the corresponding sites of $pMFT7H_6$ to yield $CP1_{Leu}$ with an N-terminal His_6 tag. The sequence of the $CP1_{Leu}$ was confirmed by DNA sequencing (data not shown). Gene expression was carried out with the method described by Chen (*27*).

Purification of His_6-Tagged Proteins. Purification of His_6-tagged LeuRS (native), LeuRS-B, and $CP1_{Leu}$ was performed with Ni-NTA Superflow (from Qiagen), as previously described (*27*). The His-tagged LeuRS was shown to have specificity and kinetic constants almost identical to those of untagged native LeuRS (*27*).

Assay of Enzyme Activity. Activities of LeuRS were measured according to the methods described by Li et al. (*28*). The ATP−PP_i exchange activity was assayed at 37 °C in the reaction mixture containing 100 mM HEPES (pH 7.8), 10 mM $MgCl_2$, 10 mM KF, 4 mM ATP (from Sigma), 2 mM [^{32}P]pyrophosphate (from Amersham), an appropriate amount of amino acid (from Sigma), and enzymes (purified in our lab). The kinetics of ATP−PP_i exchange reaction was assayed with 4 nM LeuRS or 8 nM LeuRS-B, 5−50 mM methionine or isoleucine, or 0.02−0.2 mM leucine. The aminoacylation activity was determined at 37 °C in the reaction mixture consisted of 100 mM Tris-HCl (pH 7.8), 30 mM KCl, 12 mM $MgCl_2$, 4 mM ATP, 0.1 mM EDTA, 0.5 mM DTT, 20 μM $tRNA^{Leu}$ (isolated from an overexpression strain constructed in our lab) (*29*), an appropriate amount of ^{14}C-labeled amino acids (from Amersham), and enzymes. One unit of aminoacylation activity was defined as the amount of enzyme that charges 1 nmol of $tRNA^{Leu}$ per minute under the given condition. The kinetic constants of enzymes were determined using various concentrations of the relevant substrates. To assay the mischarging of noncognate amino acids, L-[^{14}C]methionine or L-[^{14}C]isoleucine (from Amersham) was used in the aminoacylation activity assay in place of the cognate substrate L-[^{14}C]leucine. The concentration of purified LeuRS and LeuRS-B was measured by optical absorbancy at 280 nm. At this wavelength, 1 unit of optical density correlates to about 1.60 mg/mL LeuRS, or 1.57 mg/mL His_6-tagged LeuRS-B (*30*).

Circular Dichroism (CD) Spectroscopy. Protein samples at the concentration of 0.20 mg/mL were analyzed on Jasco J-715 spectropolarimeter with a nitrogen purge at room temperature. A 0.1 cm path length cuvette was used, and spectra were accumulated over five scans. Estimation of the secondary structure by the CD spectrum was calculated according to the method of Yang (*31*).

RESULTS

Purification of E. coli LeuRS-B and $CP1_{Leu}$. The recombinant plasmid containing the genes encoding LeuRS-B or $CP1_{Leu}$ was obtained. The genes were overexpressed in *E. coli* JM109(DE3). After one-step affinity chromatography on Ni-NTA Superflow, LeuRS-B and $CP1_{Leu}$ with the correct molecular masses were purified to a single band on SDS−PAGE (Figure 2). LeuRS was purified in the same way as a control. The specific activity of LeuRS-B for aminoacylation was 790 units/mg, which is 50% of that of LeuRS.

Comparison of CD Spectra of LeuRS, LeuRS-B, and $CP1_{Leu}$. To determine whether the insertion mutation alters

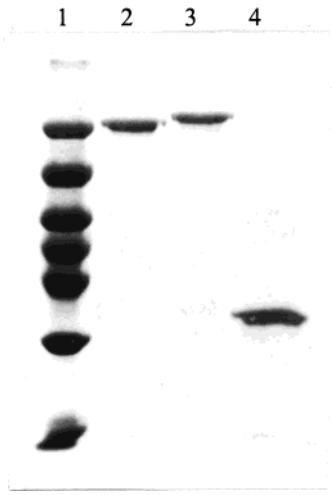

FIGURE 2: SDS−PAGE analysis of purified *E. coli* LeuRS, LeuRS-B, and $CP1_{Leu}$. Polyacrylamide gel electrophoresis with a 10% sepatrating gel and a 4% stacking gel was carried out in the presence of sodium dodecyl sulfate (SDS−PAGE). Proteins were visualized by Coomassie blue staining: lane 1, protein standards with molecular masses of 97.4, 66.2, 55.0, 42.7, 40.0, 31.0, and 21.5 kDa (from top to bottom), respectively; lanes 2−4, 5 μg of *E. coli* LeuRS, LeuRS-B, and $CP1_{Leu}$ purified by one-step Ni-NTA Superflow chromatography, respectively.

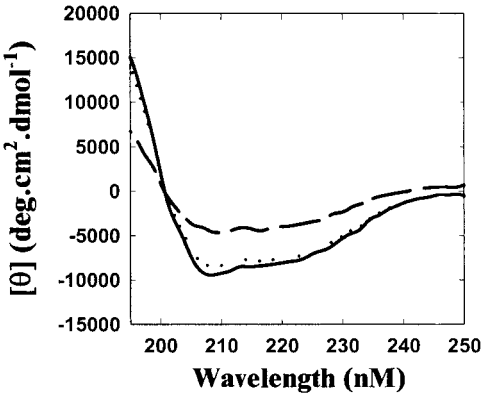

FIGURE 3: CD spectra of LeuRS, LeuRS-B, and $CP1_{Leu}$. The CD spectra of *E. coli* LeuRS (—), LeuRS-B (···), and $CP1_{Leu}$ (− − −) were accumulated over five scans. The concentration of the proteins was 0.20 mg/mL. A 0.1 cm path length cuvette was used.

the secondary structure of LeuRS and whether $CP1_{Leu}$ has a definite secondary structure, the CD spectra of LeuRS, LeuRS-B, and $CP1_{Leu}$ were measured. The CD spectra of LeuRS and LeuRS-B appeared to be almost identical, indicating that the insertion did not significantly affect the secondary structure of LeuRS. The $CP1_{Leu}$ was also shown to have a definite secondary structure (Figure 3). The parameters of their secondary structures were estimated by the method of Yang and summarized in Table 1.

Aminoacylation Kinetics of E. coli LeuRS and LeuRS-B. To analyze the functional alteration of *E. coli* LeuRS caused by the insertion mutation within CP1 domain, we measured the kinetic constants for the aminoacylation reaction of *E. coli* LeuRS and LeuRS-B (Table 2). The insertion mutation induced a 50% decline in the values of k_{cat} for leucine, ATP, and $tRNA^{Leu}$ compared to those of the native enzyme. The K_m values for leucine and ATP were similar between *E. coli* LeuRS and LeuRS-B, but the K_m value for $tRNA^{Leu}$ was more significantly affected by the insertion (1.5 μM for LeuRS and 2.4 μM for LeuRS-B), indicating that the insertion mutation has little effect on the binding of leucine and ATP, but causes looser binding of $tRNA^{Leu}$ on the enzyme. These results also suggested that the CP1 domain in *E. coli* LeuRS might be involved in $tRNA^{Leu}$ binding.

Table 1: CD Estimates of LeuRS, LeuRS-B, and $CP1_{Leu}$[a]

protein secondary structure	LeuRS	LeuRS-B	$CP1_{Leu}$
α-helix	38.2%	37.0%	24.4%
β-sheet	34.3%	33.7%	52.2%
β-turn	0.0%	2.3%	0.0%
random	27.5%	27.0%	23.5%

[a] All spectra are averages of five scans at room temperature. A 0.1 cm optical path length cuvette and enzymes at concentrations of 0.20 mg/mL were used. Estimation of the secondary structure with CD spectra was calculated according to the method of Yang (*31*).

Table 2: Aminoacylation Kinetic Constants of LeuRS and LeuRS-B[a]

substrate	constant	LeuRS	LeuRS-B
leucine	K_m (μM)	15	15
	k_{cat} (s^{-1})	3.0	1.5
	k_{cat}/K_m (s^{-1} mM^{-1})	202	100
ATP	K_m (μM)	280	250
	k_{cat} (s^{-1})	3.6	1.6
	k_{cat}/K_m (s^{-1} mM^{-1})	12.9	6.4
$tRNA^{Leu}$	K_m (μM)	1.5	2.4
	k_{cat} (s^{-1})	2.9	1.3
	k_{cat}/K_m (s^{-1} mM^{-1})	1933	542

[a] Aminoacylation kinetics of enzymes were determined as described in Experimental Procedures. All the data in this table were the average values with a variation of <5% from three independent determinations.

Table 3: Kinetic Constants of Activation and Misactivation by LeuRS and LeuRS-B[a]

enzymes	substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})	discrimination factor $(k_{cat}/K_m)_{Leu}/(k_{cat}/K_m)_{Met\ or\ Ile}$
LeuRS	Leu	0.052	171	3.3×10^3	
	Met	7.5	19	2.5	1.3×10^3
	Ile	3.5	18	5.1	6.5×10^2
LeuRS-B	Leu	0.069	101	1.5×10^3	
	Met	6.2	7.6	1.2	1.3×10^3
	Ile	2.8	6.9	2.5	6.0×10^2

[a] ATP−PP_i exchange kinetics of enzymes were determined in the presence of 0.02−0.2 mM leucine and 5−50 mM methionine or isoleucine. All the data in this table were the average values with a variation of <10% from three independent determinations. The discrimination factors were calculated from the equation $D_{Met\ or\ Ile} = (k_{cat}/K_m)_{Leu}/(k_{cat}/K_m)_{Met\ or\ Ile}$.

Discrimination between Cognate and Noncognate Amino Acids by LeuRS and LeuRS-B. To evaluate the contribution of the CP1 domain to the discrimination between leucine and methionine or isoleucine in the amino acid activation reaction (the coarse sieve), ATP−PP_i exchange kinetic constants of LeuRS and LeuRS-B were measured in the presence of 0.02−0.2 mM leucine and 5−50 mM methionine or isoleucine (Table 3). The discrimination factor D can be calculated from kinetic constants with the equation $D = (k_{cat}/K_m)_{Leu}/(k_{cat}/K_m)_{Met\ or\ Ile}$. The values of LeuRS-B for methionine and isoleucine were shown to be the same as those of LeuRS, demonstrating that amino acid discrimination was not affected by the insertion and implying that the CP1 domain in *E. coli* LeuRS is not the structural basis of the discrimination

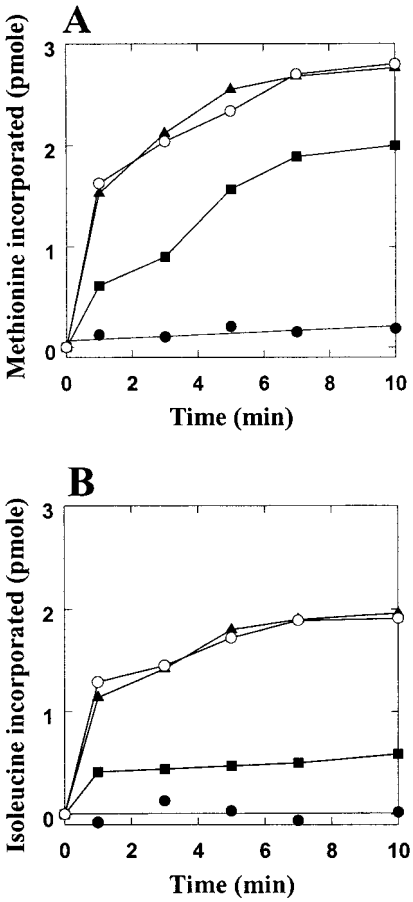

FIGURE 4: Methionylation and isoleucylation of $tRNA^{Leu}$ by *E. coli* LeuRS and LeuRS-B. (A) Methionylation of 20 μM $tRNA^{Leu}$ in the presence of 1 mM [^{14}C]methionine by 750 nM *E. coli* LeuRS (●), 750 nM LeuRS-B (▲), 750 nM LeuRS-B and 35 nM LeuRS (■), and 750 nM LeuRS-B and 10 μM $CP1_{Leu}$ (○) at 37 °C and pH 7.8. (B) Isoleucylation at pH 7.8 and 37 °C of 20 μM $tRNA^{Leu}$ in the presence of 1 mM [^{14}C]isoleucine by 750 nM *E. coli* LeuRS (●), 750 nM LeuRS-B (▲), 750 nM LeuRS-B and 35 nM LeuRS (■), and 750 nM LeuRS-B and 10 μM isolated $CP1_{Leu}$ (○).

between cognate and noncognate amino acids in the first reaction (coarse sieve).

Misaminoacylation of $tRNA^{Leu}$ Edited by LeuRS and LeuRS-B. To reveal the function of the CP1 domain in *E. coli* LeuRS during editing (the fine sieve), the aminoacylation of $tRNA^{Leu}$ was assayed in the presence of 1 mM [^{14}C]-methionine or [^{14}C]isoleucine instead of 0.1 mM [^{14}C]leucine (Figure 4). It appeared that $tRNA^{Leu}$ could be methionylated or isoleucylated by *E. coli* LeuRS-B but not LeuRS, indicating that LeuRS-B had an impaired editing function as a fine sieve for correcting the errors in aminoacylation reaction, which was brought about by the insertion of 41 amino acid residues in the CP1 domain and disturbed the interaction between CP1 and $tRNA^{Leu}$. The misacylation of $tRNA^{Leu}$ by methionine and isoleucine could be partially corrected by addition of 35 nM native LeuRS to the reaction mixture, but not 10 μM (286 times more concentrated than LeuRS) isolated wild-type CPl_{Leu} (Figure 4). These data indicate that the CP1 domain is crucial for fine sieve editing function of *E. coli* LeuRS and may not function independently but is only able to perform its editing function in the context of the enzyme.

A competition experiment of methionine or isoleucine for leucine was also performed to confirm the observation

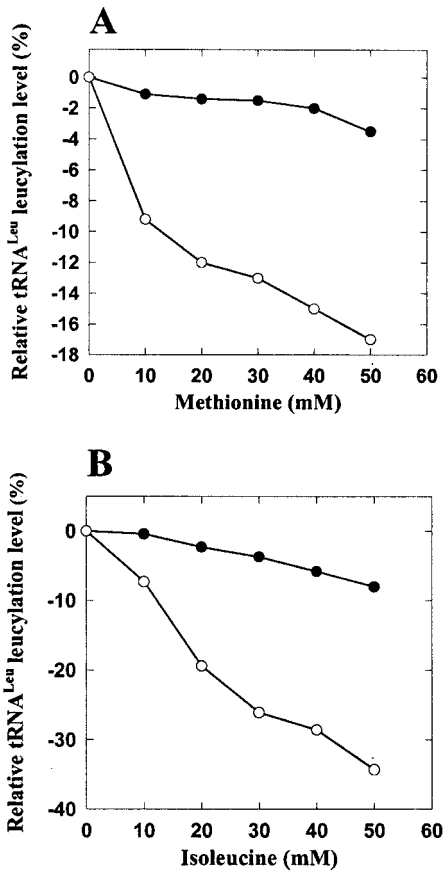

FIGURE 5: Leucylation of $tRNA_{Leu}$ by *E. coli* LeuRS and LeuRS-B with methionine or isoleucine as a competitor against [^{14}C]leucine. The *Y*-axis shows the percentage of leucylation activity decrease in the presence of methionine or isoleucine compared to that without the competitive amino acid. (A) Leucylation at pH 7.8 and 37 °C of 20 μM $tRNA_{Leu}$ by *E. coli* LeuRS (●) and LeuRS-B (○) with 10−50 mM methionine and 0.1 mM [^{14}C]leucine. (B) Leucylation at pH 7.8 and 37 °C of 20 μM $tRNA_{Leu}$ by *E. coli* LeuRS (●) and LeuRS-B (○) with 10−50 mM isoleucine and 0.1 mM [^{14}C]leucine.

described above. Aminoacylation activity of LeuRS and LeuRS-B was measured in the standard buffer containing 0.1 mM [^{14}C]leucine with the addition of methionine or isoleucine at concentrations ranging from 10 to 50 mM, which will compete with leucine for activation and transfer to $tRNA^{Leu}$. These misactivation and mischarge, if not corrected during the editing step, will decrease the [^{14}C]-leucine-$tRNA^{Leu}$ yield and hence the apparent leucylation activity, as compared to that in the absence of Met and Ile. Indeed, the leucylation activity of the mutant was decreased when methionine or isoleucine was added to the aminoacylation reaction system (Figure 5). In the presence of 50 mM methionine or isoleucine, the leucylation activity of LeuRS-B decreased to about 83 or 66%, respectively, as compared with the activity in the absence of the competitors. However, the leucylation activity of the native enzyme in the presence of 50 mM methionine or isoleucine decreased only 3.5 and 8% compared to that observed in the absence of competitor. In the case of the native enzyme, the decrease was probably caused by trace contamination of leucine in the methionine and isoleucine preparation (*3*). These results further confirm that LeuRS-B has an impaired fine sieve editing function for correcting the errors in the aminoacylation reaction, which suggests that the CP1 domain is crucial for fine sieve editing function of *E. coli* LeuRS.

DISCUSSION

This is the first report of the mechanism of the editing function of *E. coli* LeuRS. In this presentation, an insertion mutation in the CP1 domain of *E. coli* LeuRS was constructed. Although the insertion mutant retained 50% leucylation activity of the native enzyme, it lost part of the ability to discriminate other noncognate amino acids from leucine and caused mischarging of $tRNA^{Leu}$. LeuRS-B retained the "coarse sieve" discrimination of amino acids in the activation reaction, but was greatly impaired in fine sieve editing function. Therefore, the coarse and the fine sieves are located at different sites in *E. coli* LeuRS. Since this mutant could not deacylate the incorrectly charged $tRNA^{Leu}$, the level of errors in aminoacylation was significantly increased. The errors could be corrected by the intact native *E. coli* LeuRS but not by the isolated $CP1_{Leu}$, indicating that the CP1 domain in *E. coli* LeuRS serves as the fine sieve for editing but needs to be in the correct context of the synthetase to function properly. Currently, no crystal structure is available for LeuRS. Our data show that the exact primary structure of the CP1 domain of LeuRS is necessary for editing in *E. coli* LeuRS as a fine sieve and provide strong support for the role of the CP1 domain in the editing function. Also, it provides more details on the structural basis of the functional difference between the CP1 domains of the relative synthetase in the same subclass.

Among the 10 class I aaRSs, LeuRS, ValRS, IleRS, MetRS, and CysRS are more closely related to each other and are classified in one subgroup of class I aaRSs (*20*). MetRS and CysRS have relatively small CP1 domains (100 and 50 amino acids, respectively) with no known editing activity. The other three (LeuRS, ValRS, and IleRS) have large CP1 domains. The CP1 of ValRS from *B. stearothermophilus* consists of 221 amino acid residues, while those from IleRS and MetRS from *E. coli* are 275 and 106, respectively (*21*). Previous work of cloning and expression of DNA fragments that encode only the CP1 domain has shown that the isolated $CP1_{Val}$ domain from *B. stearothermophilus* ValRS and $CP1_{Ile}$ from *E. coli* IleRS harbor the editing function of deacylating Thr-$tRNA^{Val}$ and Val-$tRNA^{Ile}$, respectively (*22*). The $CP1_{Leu}$ (260 residues) was stable during the purification procedure and had a definite secondary structure. Even though the CP1 domains from these three synthetases (IleRS, ValRS, and LeuRS) are so closely related, the isolated $CP1_{Leu}$ has no editing function while the isolated $CP1_{Val}$ and $CP1_{Ile}$ can. Therefore, the $CP1_{Leu}$ serves as the fine sieve for editing only in the intact LeuRS. The editing mechanism of CP1 in *E. coli* LeuRS may differ from that of ValRS from *B. stearothermophilus* and IleRS from *E. coli*.

From an evolutionary viewpoint, the ancestor of LeuRSs might catalyze activation of several similar amino acids, such as leucine, isoleucine, methionine, and valine. Divergence of this ancestral gene by duplication and mutation could gradually generate a series of improved synthetases that could catalyze the charging of cognate amino acids with greater specificity. Among these enzymes, MetRS developed a coarse sieve that could very efficiently discriminate other similar amino acids from its cognate substrate in the amino acid activation reaction so that the editing function was no longer required and finally disappeared. However, for LeuRSs, IleRSs, and ValRSs, the coarse sieve discrimination is insufficient and the editing (by the CP1 domain) is necessary to maintain the accuracy of the aminoacylation reaction. This hypothesis is supported not only by the results from our lab and others (*24*) but also by the comparison of the codons encoding the amino acids: CTX or TTA(G), leucine; ATA(C,T), isoleucine; ATG, methionine; and GTX, valine (where X is a wobble base). The codons for the four amino acids mentioned above are similar. It could be proposed that the XTX codon is shared by leucine, isoleucine, methionine, and valine in an ancient aminoacylation enzyme system.

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