Discrimination of $tRNA^{Leu}$ Isoacceptors by the Mutants of *Escherichia coli* Leucyl-tRNA Synthetase in Editing†

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ABSTRACT: Leucyl-tRNA synthetase (LeuRS), one of the class Ia aminoacyl-tRNA synthetases, joins Leu to $tRNA^{Leu}$ and excludes noncognate amino acids in protein synthesis. In this study, *Escherichia coli* LeuRS mutants at amino acid E292, which was located in the connective polypeptide 1 insertion region, were synthesized. Although mutated LeuRS showed little change in structure compared with wild-type LeuRS, the mutants were impaired in activity to varying extents. It was also showed that mutations did not affect the adenylation reaction. However, mutated LeuRS can mischarge $tRNA^{Leu}$ isoacceptors $tRNA_1^{Leu}$ or $tRNA_2^{Leu}$ with isoleucine to different extents. Isoleucylation of $tRNA_1^{Leu}$ was more than that of $tRNA_2^{Leu}$. The mutant LeuRS-E292S, which was picked out as an example for the investigation of the relationship between $tRNA^{Leu}$ isoacceptors and editing function, can discriminate the Watson−Crick base pair of the first base pair of $tRNA^{Leu}$ from the wobble base pair. The $tRNA^{Leu}$ with the Watson−Crick base pair may result in more isoleucylated product than that with the wobble base pair. The same phenomenon happened to another mutant, LeuRS-A293D. It seems that the flexibility of the first base pair affects the editing reaction of LeuRS. The results indicate that the flexibility of the first base pair of $tRNA^{Leu}$ may probably affect the mischarged 3′-end of $tRNA^{Leu}$ shuttling from synthetic site to editing site and that the transferred acceptor arm of $tRNA^{Leu}$ may interact with LeuRS in the region around E292.

Aminoacyl-tRNA synthetases (aaRSs)[1] (EC 6.1.1) catalyze the reaction for attaching specific amino acids to their cognate tRNAs (*1*−*3*). Each aaRS must be able to precisely distinguish its cognate substrates, amino acids, and tRNAs from many substrates with similar structure (*4*). The 20 aaRSs are divided into two classes, of 10 members each, on the basis of their conserved sequences and characteristic structural motifs (*5*). The active sites of class I aaRSs are based on the Rossmann fold (an overall $\beta_6\alpha_4$ structure) (5), which is made up of two $\beta_3\alpha_2$ halves, linked by the connective polypeptide 1 (CP1) (*6*, *7*).

In general, the aminoacylation of tRNA is a two-step reaction: (a) the activation of amino acids with adenosine triphosphate (ATP) by forming aminoacyl adenylates and (b) the transferring of the aminoacyl residue from aminoacyl adenylate to the cognate tRNA substrate (*8*). The accuracy of aminoacylation depends on both the specific recognition of amino acids during their activation (coarse sieve) and the pre- or posttransfer editing (fine sieve) that corrects errors at either the aminoacyl adenylate level or the tRNA level (*9*−*11*). These editing reactions during the aminoacylation of tRNAs by aaRSs are essential for the accurate incorporation of amino acids during protein biosynthesis (*10*, *12*−*14*).

Leucyl-tRNA synthetase (LeuRS, EC 6.1.1.4) from *Escherichia coli* (*E. coli*) is a single peptide enzyme consisting of 860 amino acid residues with a putative molecular mass of 97.3 kDa, deduced from the leuS gene sequence (*15*). LeuRS, valyl-tRNA synthetase (ValRS), isoleucyl-tRNA synthetase (IleRS), methionyl-tRNA synthetase (MetRS), and cysteinyl-tRNA synthetase (CysRS) belong to a subgroup of class I aaRSs (*16*). LeuRS, IleRS, and ValRS are closely related to the large monomer class I synthetases, as each contains a homologous CP1 domain of about 200 residues (*6*). The crystal structure of *Thermus thermophilus* (*T. thermophilus*) IleRS revealed that the CP1 domain contains a serine protease-like fold, which is a putative editing active site that hydrolyzes cognate tRNAs that have been mischarged with a chemically similar but noncognate amino acid (*17*). A crystal structure of *Staphylococcus aureus* (*S. aureus*) IleRS complexed with an *E. coli* $tRNA^{Ile}$ transcript and an inhibitor revealed the first putative posttransfer editing complex. In this structure, the 3′-end of the tRNA was directed toward the putative editing active site rather than the aminoacylation active site (*18*). Similarly, the 3′-terminal strand of $tRNA^{Val}$ extended straight to the CP1 domain of ValRS from *T. thermophilus* (*19*). The crystal structure of *T. thermophilus* LeuRS complexed with a leucyl adenylate

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[1] Abbreviations: aaRSs, aminoacyl-tRNA synthetases; CP1, connective polypeptide 1; *E. coli*, *Escherichia coli*; *T. thermophilus*, *Thermus thermophilus*; *S. aureus*, *Staphylococcus aureus*; LeuRS, leucyl-tRNA synthetase; ValRS, valyl-tRNA synthetase; IleRS, isoleucyl-tRNA synthetase; MetRS, methionyl-tRNA synthetase; CysRS, cysteinyl-tRNA synthetase; DTT, dithiothreitol.

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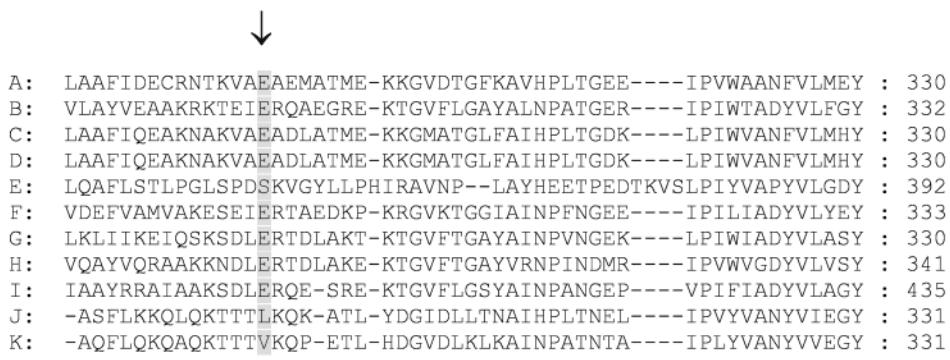

FIGURE 1: Primary sequence alignment of LeuRS from different organisms: A = *E. coli*, B = *T. thermophilus*, C = *Haemophilus influenzae*, D = *Borrelia burgdorfer*, E = *Neurospora crassa* (mit), F = *Synechocystis* sp., G = *B. subtilis*, H = *Treponema pallidum*, I = *Mycobacterium tuberculosis*, J = *Mycoplasma genitalium*, and K = *Mycoplasma pneumoniae*. Alignment corresponding to E292 of the *E. coli* enzyme is shaded and indicated by an arrow. This sequence alignment was extracted from an alignment built with the program CLUSTAL X.

analogue is similar to that of IleRS, except that the putative editing domain is inserted at a different position in the primary structure (*20*). On the basis of the structure of *T. thermophilus* LeuRS and sequence alignments of the aaRSs in the same subgroup, CP1 of *E. coli* LeuRS extends from residue 126 to residue 418 (*20*, *21*).

We found that the peptide bond between E292−A293 in CP1 of *E. coli* LeuRS was specifically cleaved (*22*). The cleaved LeuRS retained the ATP−PP_i exchange activity; however, the aminoacylation activity was lost (*22*). This peptide bond seems to be in a very special location in the CP1 domain of this enzyme and appears to be very important for aminoacylation activity. On the basis of the crystal structure of *T. thermophilus* LeuRS and the alignment results, E292 and A293 were located in a small helix of the CP1 domain of the enzyme and on the side facing the active site (*20*). Replacement of the A293 residue with any other amino acid leads to impaired activity and editing (*23*). An insertion mutant, LeuRS from *E. coli*, with a duplication of the peptide fragment from Met328 to Pro368 within the CP1 domain, LeuRS-B, is impaired in its ability to edit incorrect products (*24*). It has also been reported that T252 of *E. coli* LeuRS prevents hydrolytic editing of leucyl-$tRNA^{Leu}$ (*25*). Therefore, the CP1 domain is a very important domain for the catalytic and editing activities of *E. coli* LeuRS.

Sequence alignment of LeuRSs from different organisms showed that the E292 is highly conserved; eight of the eleven amino acids are glutamic acid and the other three residues are serine, leucine, and valine, respectively (Figure 1). To further reveal the effect of E292 on *E. coli* LeuRS, six mutations in E292 of LeuRS were introduced. In this paper, we report the declined aminoacylation and editing activity of the mutants, discrimination of $tRNA^{Leu}$ isoacceptors in editing by the mutants, and the effect of the first base pair in the acceptor stem of $tRNA^{Leu}$ to the editing function of LeuRS.

EXPERIMENTAL PROCEDURES

Materials. L-Leucine, ATP, DTT, DEAE-Sepharose CL-6B fast flow, and HA-Ultragel were purchased from Sigma. [^{32}P]Pyrophosphate was from NEN Life Sciences Products. L-[^{14}C]Leucine and L-[^{14}C]isoleucine were purchased from Amersham (England). Enzymes were products of MBI Ferments or Promega. The purified *E. coli* $tRNA_1^{Leu}$ and $tRNA_2^{Leu}$ expressed in vivo, which have a charging capacity of about 1400 pmol/A_{260} unit (*26*), were used to test mischarging with noncognate amino acids. T7 RNA polymerase was purified from an *E. coli* strain carrying a T7 RNA polymerase overproducing plasmid (*27*), a gift from H. Asahara. The gene encoding *E. coli* LeuRS, leuS, was cloned in our laboratory (*28*). The plasmid pTrc-99B (*29*) was a gift from J. Gangloff. *E. coli* LeuRS was purified from the *E. coli* overproducing strain constructed in our laboratory (*30*).

Mutagenesis, Expression, and Purification of LeuRS and Its Mutants. The genes encoding the six LeuRS mutants changed to K, F, S, D, Q, and A at amino acid E292 were amplified by PCR, inserted into the plasmid pTrc99B, and expressed as previously described (*23*). The DNA sequence fidelities of the mutants were confirmed by DNA sequencing (data not shown). LeuRS and its mutants were purified from overproducing *E. coli* TG1 transformants by two-step chromatography on DEAE-Sepharose CL-6B fast flow and HA-Ultragel (*30*). Protein concentration was measured by Bradford protein assay (*31*).

Assay of Enzyme Activity. Aminacylation activity and kinetic constants were assayed as before (*22*). The ATP−PP_i exchange reaction and kinetic constants were measured according to the protocol of Chen et al. (*24*). Misaminoacylation activity was determined at 37 °C in a reaction mixture consisting 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, 4 μM $tRNA^{Leu}$ (from in vitro transcription) or 6 μM $tRNA^{Leu}$ (isolated from overproduced strain; *26*), 1 mM [^{14}C]-isoleucine, and 5 μM LeuRS. The reaction was initiated with the addition of LeuRS. At varying time intervals (2, 4, 7, 11, and 15 min), 10 μL aliquots were applied to Whatman 3MM filters and treated as previously described for the aminoacylation reaction (*22*).

Circular Dichroism (CD) Spectroscopy. Protein samples (0.20 mg/mL) in 10 mM potassium phosphate buffer (pH 6.8) were measured on a Jasco J-715 spectropolarimeter at room temperature. A 0.1 cm path-length cuvette was used, and spectra were accumulated over four scans.

Fluorescence Emission Spectroscopy. Fluorescence emission spectra were recorded with a Hitachi F4010 fluorescence spectrophotometer. Protein samples (0.20 mg/mL) in 10 mM potassium phosphate buffer (pH 6.8) were excited at 295 nm (slit 3 nm), and emission spectra were recorded in the 300−400 nm range.

Table 1: Specific Activities of LeuRS and Its Mutants at E292[a]

enzyme	specific activity (units/mg of protein)	relative activity (%)
LeuRS	2703 ± 50	100
LeuRS-E292D	1270 ± 45	47
LeuRS-E292Q	1243 ± 60	46
LeuRS-E292F	1080 ± 25	40
LeuRS-E292A	1054 ± 32	39
LeuRS-E292S	919 ± 23	34
LeuRS-E292K	405 ± 20	15

[a] The values are the mean (± standard error) of three independent determinations.

Transcription of tRNA in Vitro. The plasmid carrying the T7 promoter and the $tRNA_1^{Leu}$ gene was a gift from H. Asahara (*32*). The genes encoding the mutants of $tRNA_1^{Leu}$ and $tRNA_2^{Leu}$ were cloned previously (*26*). The tRNA was transcribed in a reaction mixture containing 40 mM Tris-HCl (pH 8.0), 5 mM DTT, 10 mM $MgCl_2$, 2 mM NTPs, 8 mM 5′-GMP (Sigma), *Bst*OI-digested template DNA (50 nM), 1 unit/mL inorganic pyrophosphatase (Sigma), and 400 μg/mL T7 RNA polymerase. The transcripts were purified by 20% (w/v) denaturing polyacrylamide gel electrophoresis (*33*).

RESULTS

Expression and Purification of LeuRS and Its Mutants. The genes encoding the six mutants of LeuRS replaced by other amino acid residues at E292 were obtained. The six mutants were overproduced at similar levels in *E. coli*. LeuRS and the six single-site mutants at E292 were purified by two-step chromatography as described previously (*30*). Their specific aminoacylation activities are shown in Table 1. All mutants at position 292 were decreased significantly in activity to different extents. Especially, the LeuRS-E292K exhibited the greatest reduction (85%), while LeuRS-E292D exhibited the least reduction (53%). This means that the charge conversion at E292 of LeuRS will result in the largest loss in aminoacylation activity.

Comparison of CD Spectra and Fluorescence Emission Spectra of LeuRS and Its Mutants. To gain insight into whether the impaired aminoacylation activity of the mutated LeuRSs resulted from the conformation change, spectra of CD and fluorescence were determined. CD spectra showed that there were no detectable changes on secondary structure between LeuRS and its mutants (data not shown). The CD spectra of LeuRS and LeuRS-E292K (which was selected because of its lowest aminoacylation activity) are demonstrated in Figure 2, and the two curves were almost the same. The fluorescence emission spectra are shown in Table 2. The data showed that the center of mass and fluorescence emission maximum of LeuRS and its mutants were almost unchanged. Hence, the substitution of E292 of LeuRS might affect the subtle conformation of LeuRS.

Discrimination between Cognate and Noncognate Amino Acids by LeuRS and the Six E292 Mutants. Although the conformation was not changed in mutated LeuRSs, the substitution of E292 of LeuRS resulted in decreasing aminoacylation activity. Does such substitution affect the first step reaction (activation reaction)? We found that, in the first step reaction, the specific activity of LeuRS and its six mutants was almost the same (data not shown). Therefore,

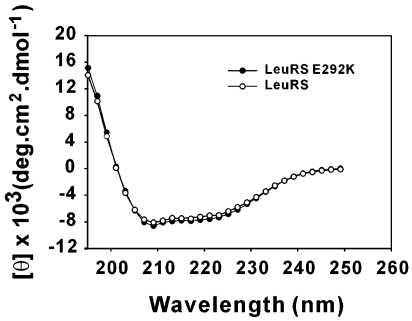

FIGURE 2: Circular dichroism spectra of LeuRS (○) and LeuRS-E292K (●). The concentration of the proteins was 0.20 mg/mL. A 0.1 cm path-length cuvette was used.

Table 2: Fluorescence Emission Spectra Parameters of LeuRS and Its Mutants[a]

enzyme	emission wavelength max (nm)	fluorescence intensity max	center of mass (cm^{-1})
LeuRS	338.4	117.4	29233
LeuRS-E292K	338.4	119.5	29234
LeuRS-E292F	339.0	120.3	29242
LeuRS-E292S	339.0	115.3	29231
LeuRS-E292D	338.2	118.0	29230
LeuRS-E292Q	339.0	119.3	29231
LeuRS-E292A	339.2	116.2	29230

[a] Protein samples (0.2 mg/mL) in 10 mM potassium phosphate buffer (pH 6.8) were excited at 295 nm (3 nm slit), and emission spectra were recorded in the 300−400 nm range.

Table 3: Kinetic Constants of Activation and Misactivation by LeuRS and LeuRS-E292S

enzyme	substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}\,mM^{-1}$)	$(k_{cat}/K_m)_{Leu}/(k_{cat}/K_m)_{Ile}$[a]
LeuRS	Leu	0.034	180	5.3×10^3	1
	Ile	3.5	15	4.3	1.2×10^3
LeuRS-E292S	Leu	0.034	154	4.5×10^3	1
	Ile	3.7	14	3.8	1.2×10^3

[a] Discrimination factor.

the single-site substitution mutation at E292 did not affect leucine activation. To evaluate the contribution of E292 to the discrimination between leucine and isoleucine in the amino acid activation reaction (the coarse sieve), ATP−PP_i exchange kinetic constants of LeuRS and LeuRS-E292S were measured in the presence of 0.02−0.2 mM leucine and 5−50 mM isoleucine. The discrimination factor *D* of LeuRS-E292S for isoleucine, which can be calculated from kinetic constants by the equation $D = (k_{cat}/K_m)_{Leu}/(k_{cat}/K_m)_{Ile}$, was shown to be nearly the same as that of LeuRS (Table 3), thus demonstrating that amino acid discrimination was not affected by the substitution. This implies that E292 in *E. coli* LeuRS is not involved in the discrimination between cognate and noncognate amino acids in the first reaction.

Editing of Misaminoacylation of $tRNA^{Leu}$ by LeuRS and E292 Mutants. As we know, the CP1 domain in *E. coli* LeuRS is crucial for its editing function (*23*−*25*). To gain insight into the role of E292 in the editing function of the CP1 domain in *E. coli* LeuRS, under the same conditions, the misaminoacylation of two isoacceptors, $tRNA_1^{Leu}$ and $tRNA_2^{Leu}$ purified in vivo (*26*) was assayed in the presence of 1 mM [^{14}C]isoleucine (Figure 3). The results demonstrated that both $tRNA_1^{Leu}$ and $tRNA_2^{Leu}$ could be isoleucylated by all of the E292 amino acid substitution mutants, but not by

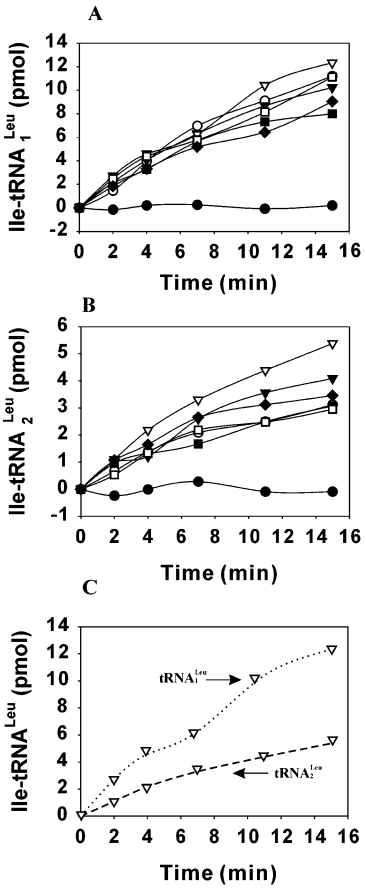

FIGURE 3: Isoleucylation of tRNA^{Leu} isoacceptors by *E. coli* LeuRS and its mutants at E292. Isoleucylation at 37 °C, pH 7.8, of 6 μM tRNA^{Leu} in the presence of 1 mM [^{14}C]isoleucine by 5 μM *E. coli* LeuRS (●), 5 μM LeuRS-E292D (■), 5 μM LeuRS-E292Q (□), 5 μM LeuRS-E292A (◆), 5 μM LeuRS-E292K (○), 5 μM LeuRS-E292F (▼), and 5 μM LeuRS-E292S (▽). (A) Isoleucylation of tRNA$_1^{Leu}$. (B) Isoleucylation of tRNA$_2^{Leu}$. Panel C shows the different level in isoleucylation of tRNA$_1^{Leu}$ (···) and tRNA$_2^{Leu}$ (---) by LeuRS-E292S.

LeuRS, indicating that a single amino acid substitution at E292 could impair the editing function for correcting errors in the aminoacylation reaction. Notably, the isoleucylation of tRNA$_1^{Leu}$ (Figure 3A) was greater than that of tRNA$_2^{Leu}$ (Figure 3B) under the same conditions (tRNA concentrations were both 6 μM in the reaction mixture). In Figure 3C, we showed the isoleucylation of tRNA$_1^{Leu}$ and tRNA$_2^{Leu}$ by LeuRS-E292S for the isoleucylation level of LeuRS-E292S was the largest among the six mutants.

Involvement of the Acceptor Stem of tRNA^{Leu} Isoacceptors in the Editing Function of LeuRS. The first base pairs of the acceptor stems of tRNA$_1^{Leu}$ and tRNA$_2^{Leu}$ are different (Figure 4). For tRNA$_1^{Leu}$, it is the standard Watson−Crick base pair G1·C72; however, for tRNA$_2^{Leu}$, it is the wobble base pair G1·U72. This leads to the question of whether the different amounts of mischarged isoleucine by LeuRS-E292S result from the difference in the first base pair of the acceptor stem of tRNA$_1^{Leu}$ and tRNA$_2^{Leu}$. To test this, tRNA$_1^{Leu}$, tRNA$_2^{Leu}$, and their mutants that exchanged their first base pair were prepared separately by in vitro transcription. In our previous work LeuRS aminoacylated these tRNA^{Leu} mutants at the same rate (*34*); in the present work LeuRS-E292S also catalyzed the aminoacylation of these tRNA^{Leu}

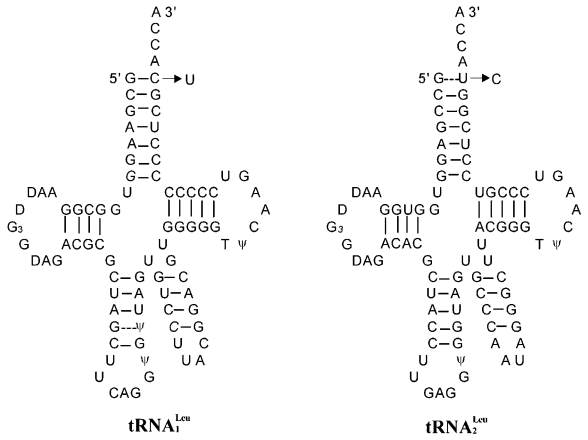

FIGURE 4: Structure of tRNA$_1^{Leu}$ and tRNA$_2^{Leu}$ and the exchange of the first base pair.

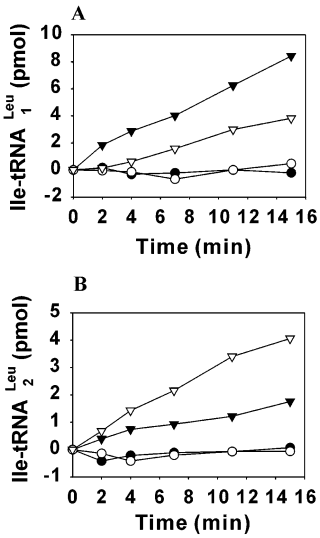

FIGURE 5: Isoleucylation of the mutants of tRNA^{Leu} by *E. coli* LeuRS-E292S: (A) in the presence of 1 mM [^{14}C]isoleucine at 37 °C, pH 7.8, isoleucylation of 4 μM tRNA$_1^{Leu}$ (●) or tRNA$_1^{Leu}$ (G1·U72) (○) by 5 μM LeuRS; isoleucylation of 4 μM tRNA$_1^{Leu}$ (▼) or tRNA$_1^{Leu}$ (G1·U72) (▽) by 5 μM LeuRS-E292S; (B) isoleucylation of 4 μM tRNA$_2^{Leu}$ (●) or tRNA$_1^{Leu}$ (G1·C72) (○) by 5 μM LeuRS; isoleucylation of 4 μM tRNA$_1^{Leu}$ (▼) or tRNA$_2^{Leu}$ (G1·C72) (▽) by 5 μM LeuRS-E292S.

mutants at the same level (data not shown). However, when the first Watson−Crick base pair of tRNA$_1^{Leu}$ was replaced by the first wobble base pair G1·U72 of tRNA$_2^{Leu}$, the isoleucyl-tRNA$_1^{Leu}$ (G1·U72) produced by LeuRS-E292S decreased (Figure 5A). When the first wobble base pair of tRNA$_2^{Leu}$ was substituted by the standard Watson−Crick base pair G1·C72 of tRNA$_1^{Leu}$, the error product, isoleucyl-tRNA$_2^{Leu}$ (G1·C72), obtained by LeuRS-E292S increased (Figure 5B). It seems, therefore, that the LeuRS-E292S can distinguish the first base pair of tRNA^{Leu} isoacceptors in the editing reaction, while the native LeuRS cannot.

Discrimination of tRNA^{Leu} Isoacceptors by Other LeuRS Mutants. Our previous research demonstrated that the LeuRS mutants at A293 (*23*) and LeuRS-B (*24*) were impaired in their editing activity. To understand whether these two mutants can distinguish the two isoacceptors of tRNA^{Leu}, isoleucylation of tRNA$_1^{Leu}$ and tRNA$_2^{Leu}$ by LeuRS-A293D and LeuRS-B, respectively, was assayed. Under the same

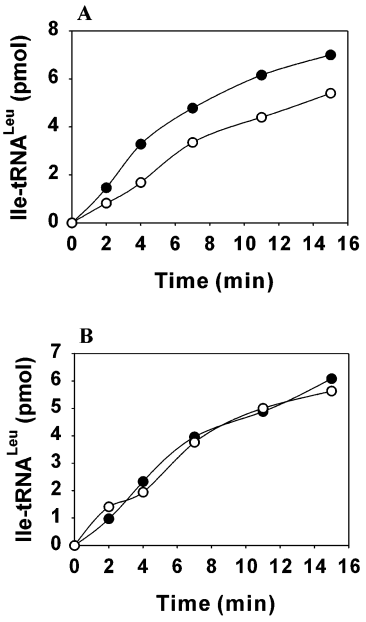

FIGURE 6: Isoleucylation of tRNA^{Leu} isoacceptors by *E. coli* LeuRS-A293D and LeuRS-B in the presence of 1 mM [^{14}C]isoleucine at 37 °C, pH 7.8: isoleucylation of 4 μM tRNA$_1^{Leu}$ (●) or 4 μM tRNA$_2^{Leu}$ (○) by 5 μM LeuRS-A293D (A) or LeuRS-B (B).

conditions, LeuRS-A293D (Figure 6A), like LeuRS-E292S, catalyzed isoleucylation of tRNA$_1^{Leu}$ more than that of tRNA$_2^{Leu}$. However, LeuRS-B catalyzed isoleucylation of tRNA$_1^{Leu}$ and tRNA$_2^{Leu}$ at almost the same rate (Figure 6B).

DISCUSSION

In the present study, the effect of E292 mutation on aminoaclyation, adenylation, and the discrimination of tRNA^{Leu} isoacceptors by mutants at A292 of *E. coli* LeuRS editing was demonstrated.

In our previous work, we demonstrated that LeuRS was attacked specifically at the peptide bond between E292 and A293. The tRNA charging activity was destroyed by cleavage of LeuRS, while leucine activation activity was almost unchanged. In vivo assembly of LeuRS cleaved between E292 and A293 was unstable, although in vivo assembly of enzymes cleaved at other sites was successful (*22*). The region around E292-A293 is most likely located on the surface of the enzyme and may play a significant role in maintaining the proper conformation of LeuRS for its function. The single-site substitution mutants at E292 impaired not only the aminoacylation active site but also the editing active site. Although the representative of the E292 single-site amino acid substitution mutants, LeuRS-E292S, retained the "coarse sieve" discrimination of amino acids in the activation reaction, it was greatly impaired in the "fine sieve" editing function in the aminoacylation reaction. The mutated LeuRS with the imperfect editing active site could distinguish the difference between the acceptor stem of tRNA$_1^{Leu}$ and tRNA$_2^{Leu}$.

From the crystal structure of *S. aureus* IleRS complexed with an *E. coli* tRNA^{Ile} transcript and an inhibitor, a shuttle mechanism of the editing of misacylated tRNA by IleRS was proposed. The amino acid editing activity of IleRS may result from the incorrect products shuttling between the synthetic and editing active sites (*18*). The data in the present work indicate that the shuttling of the 3′-end of tRNA between the synthetic and editing active sites may occur in the case of LeuRS. Isoleucylation of tRNA^{Leu} with the standard Watson−Crick base pair G1·C72 by LeuRS-E292S was more than that with the wobble base pair G1·U72. The possible reason for this may be that the standard Watson−Crick base pair is more rigid than the wobble base pair, so the movement between synthetic and editing active sites of the 3′-end of tRNA$_1^{Leu}$ is more difficult than that of tRNA$_2^{Leu}$. This hypothesis can easily explain why the tRNA$_1^{Leu}$ mutant with the wobble base pair G1·U72 was less isoleucylated due to the flexibility of the acceptor stem. For the same reason, the tRNA$_2^{Leu}$ mutant with the G1·C72 base pair was more isoleucylated by LeuRS-E292S. These results demonstrated that the editing activity of LeuRS requires not only the correct enzyme conformation but also the correct cognate tRNA structure. Furthermore, we could assume that the translocation of mischarged tRNA^{Leu} between synthetic and editing active sites of LeuRS occurs in the region around E292-A293. This is supported by the observation that LeuRS-E292S and LeuRS-A293D may recognize the difference in the first base pair of the acceptor stem of tRNA^{Leu} isoacceptor, while LeuRS-B may not.

Larkin et al. identified and compiled essential domains for tRNA^{Leu} aminoacylation and amino acid editing in *E. coli* (*35*), but the acceptor arm of tRNA^{Leu} was not investigated. Recently, Nordin and Schimmel reported that the editing sites of the other class I aaRSs, *E. coli* IleRS and ValRS, have a degree of inherent plasticity for substrate recognition. The ability to adapt to subtle differences in mischarged RNAs may be important for the high accuracy of aminoacylation (*36*). In our previous work, the data showed that an insertion mutant of LeuRS, LeuRS-A, may discriminate between two isoacceptors of tRNA^{Leu} in the aminoacylation reaction (*34*). In the present work, discrimination of tRNA^{Leu} isoacceptors by some mutants of *E. coli* LeuRS in editing is reported. These mutants were involved in E292 and A293. Crystallographic studies of these mutants can provide further insight into why these particular positions are so important.

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