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Misacylation of $tRNA^{Lys}$ with Noncognate Amino Acids by Lysyl-tRNA Synthetase†

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ABSTRACT: Lysyl-tRNA synthetase (LysRS), a class II enzyme whose major function is to provide Lys-$tRNA^{Lys}$ for protein synthesis, also catalyzes aminoacylation of $tRNA^{Lys}$ with arginine, threonine, methionine, leucine, alanine, serine, and cysteine. The limited selectivity in the tRNA aminoacylation reaction appears to be due to inefficient editing of some amino acids (Met, Leu, Cys, Ala, Thr) by a pre-transfer mechanism or the absence of editing of other amino acids (Arg and Ser). Purified Arg-$tRNA^{Lys}$, Thr-$tRNA^{Lys}$, and Met-$tRNA^{Lys}$ were essentially not deacylated by LysRS, indicating that the enzyme does not possess a post-transfer editing mechanism. However, LysRS possesses an efficient pre-transfer editing mechanism which prevents misacylation of $tRNA^{Lys}$ with ornithine. A novel feature of this editing reaction is that ornithine lactam is formed by the facile cyclization of ornithyl adenylate.

Aminoacyl-tRNA synthetases (AARSs)[1] carry out two important functions in translation: information transfer and chemical activation (*1*). The information transfer function involves matching amino acids with cognate tRNAs. Each of the 20 AARSs selects 1 cognate amino acid from over 20 biotic amino acids and a cognate tRNA from the 20 tRNA families, according to the rules of the genetic code. The chemical activation function involves aminoacylation of tRNA in a two-step reaction driven by the hydrolysis of ATP. In the first step (eq 1), a carboxyl group of an amino acid (AA) is activated by ATP, forming an AARS-bound aminoacyl adenylate:

$$\text{AARS} + \text{AA} + \text{ATP} \Leftrightarrow \text{AARS}\cdot\text{AA}\sim\text{AMP} + \text{PP}_i \quad (1)$$

In the second step (eq 2), the amino acid is transferred from the adenylate to tRNA:

$$\text{AARS}\cdot\text{AA}\sim\text{AMP} + \text{tRNA}^{AA} \Leftrightarrow \text{AARS} + \text{AA-tRNA}^{AA} + \text{AMP} \quad (2)$$

High selectivity of AARSs is a prerequisite for their pivotal role in the implementation and translation of the genetic code (*1*−*3*). When differences in binding energies of amino acids to an AARS are inadequate, editing is used as a major component of the enzyme selectivity (*4*). Editing is accomplished by destroying intermediates or products at several points along the aminoacylation pathway (*5*). In most cases, errors are removed at the stage of aminoacyl adenylate, before transfer to tRNA (*6*). Occasionally, a fraction of errors persists up to the end of the aminoacylation pathway and is then corrected by deacylation of misacylated tRNA (*7*).

Previous studies have shown that *Escherichia coli* lysyl-tRNA synthetase (LysRS) possesses an editing mechanism directed against homocysteine, homoserine, threonine, cysteine, and alanine (*8*). However, it is not known how efficient editing is in removing errors in amino acid selection by LysRS and whether some noncognate amino acids can escape editing and be incorporated into $tRNA^{Lys}$. Here it is shown that some noncognate amino acids, such as arginine, threonine, methionine, leucine, alanine, serine, and cysteine, are transferred to $tRNA^{Lys}$ whereas ornithine and homocysteine are not. A novel editing mechanism involving conversion of ornithine to ornithine lactam by LysRS is also described.

MATERIALS AND METHODS

Plasmid, Host Strain, and Culture Conditions. Escherichia coli LysRS encoded by the *lysU* gene was overexpressed from the plasmid pXLys5 (*9*) in *E. coli* strain JM101. Cells for the enzyme purification were obtained from overnight cultures (usually 400 mL, yielding ∼2 g of cells) grown at 37 °C in LB medium containing 100 μg/mL ampicillin.

Aminoacyl-tRNA Synthetases. E. coli LysRS (*8*) and ArgRS (*10*) were purified to homogeneity from overproducing strains as described before.

Radiolabeled Amino Acids. Uniformly labeled [^{14}C]Lys (316 Ci/mol), [^{14}C]Leu (308 Ci/mol), [^{14}C]Ile (308 Ci/mol), [^{14}C]Ser (151 Ci/mol), [^{14}C]Ala (150 Ci/mol), and [^{14}C]Orn (56 Ci/mol) as well as carrier-free [^{35}S]Met and [^{35}S]Cys were obtained from Amersham. [^{35}S]Hcy was prepared from [^{35}S]Met by the method of Baernstein (*11*). [^{14}C]Arg (316 Ci/mol) and [^{14}C]Thr (208 Ci/mol) were obtained from NEN. Purity of the radiolabeled amino acids was checked by TLC. No [^{14}C]Lys (<0.1%) was present in any of the radiolabeled amino acid preparations, 0.7% [^{14}C]Orn lactam was present

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[1] Abbreviations: AARS, aminoacyl-tRNA synthetase; LysRS, lysyl-tRNA synthetase; AA, amino acid; Orn, ornithine; Hcy, homocysteine; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; PAGE, polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; TLC, thin-layer chromatography.

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in the [^{14}C]Orn preparation, and 2.7% [^{14}C]Lys lactam was present in the [^{14}C]Lys preparation. These levels of lactams did not interfere with experiments.

tRNA Aminoacylations. Aminoacylation reactions were carried out at 37 °C in mixtures containing standard buffer (0.1 M K-HEPES, pH 7.4, 10 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 4 units/mL yeast inorganic pyrophosphatase (Sigma)), 2 mM ATP, 4−8 μM tRNA^{Lys} (lysine acceptor activity of 1600 pmol/A_{260}; Sigma), up to 0.4 μM AARS, and 0.01−0.25 mM [^{14}C]amino acid (56−316 Ci/mol) or [^{35}S]Hcy (10 000 Ci/mol), [^{35}S]Cys (10 000 Ci/mol). Formation of aminoacyl-tRNA^{Lys} was monitored by precipitation with 5% TCA. Aliquots of the aminoacylation mixtures were also analyzed by TLC on cellulose plates (*8*) and polyacrylamide gel electrophoresis at pH 5.5 (*12*).

Preparation of Radiolabeled Aminoacyl-tRNA^{Lys}. Reaction mixtures contained 10 μM tRNA^{Lys}, 250 μM radiolabeled amino acid, 2 mM ATP, and 0.4 μM LysRS in the standard buffer. After 1 h at 37 °C, reaction mixtures were extracted with phenol (saturated with 0.1 M sodium acetate, pH 5), and aminoacylated tRNAs were recovered from aqueous layers by precipitation with ethanol. Residual low molecular weight components from aminoacylation mixtures were removed by repeated washes with 70% ethanol. In all cases, the amounts of aminoacyl-tRNA^{Lys} purified by phenol extraction corresponded to the amounts present in the aminoacylation mixtures, as determined by TCA precipitation.

Deacylation of Radiolabeled Aminoacyl-tRNA. The radiolabeled aminoacyl-tRNA was incubated at 37 °C in the presence or absence of 2 μM LysRS in the standard buffer. The disappearance of the radiolabeled aminoacyl-tRNA was monitored by TCA precipitation. First-order rate constants, k, were calculated from reaction half-lives, $t_{0.5}$, according to $k = \ln 2/t_{0.5}$. Aliquots of reaction mixtures were also analyzed by TLC.

Editing Activity of LysRS. Unless stated otherwise, editing activity was measured as amino acid-dependent AMP formation from 0.25 mM [α-^{32}P]ATP (1000 Ci/mol) in the presence of 10−50 mM amino acid and 0.2 μM LysRS in the standard buffer (*8*). Editing of ornithine was measured from its conversion to ornithine lactam.

TLC Analyses. AMP was separated from ATP and ADP by TLC on poly(ethylenimine)−cellulose (Sigma) using 1.2 M LiCl as a solvent (*6*). Radiolabeled products formed in aminoacylation or deacylation mixtures were separated on TLC cellulose plates (from Kodak) using butanol/acetic acid/water (4:1:1, v/v) as a solvent (*8*). Nonradiolabeled amino acids, lysine lactam (Sigma), and ornithine lactam (prepared as described in ref *13*) were co-chromatoghraphed as standards and visualized by staining with ninhydrin. TLC plates were autoradiographed using Kodak BioMax MR-1 film.

RESULTS

LysRS Misacylates tRNA^{Lys} with Noncognate Amino Acids. Previous work demonstrated that LysRS edits several noncognate amino acids, such as Hcy, Cys, Thr, and Ala (*8*). Of several other amino acids tested, Met and Leu also supported the editing activity of LysRS (Table 1). Similar editing activities were also observed with norleucine, norvaline, α-aminobutyrate, and *S*-methylcysteine (not shown). To determine whether any noncognate amino acid can also be transferred to tRNA^{Lys}, aminoacylation mixtures containing radiolabeled noncognate amino acids, ATP, tRNA^{Lys}, and LysRS were prepared and analyzed by TCA precipitation (Figure 1), by TLC on cellulose plates (Figure 2), and by electrophoresis on acid polyacrylamide gels (Figure 3). Unexpectedly, tRNA^{Lys} was aminoacylated with [^{14}C]Arg, [^{14}C]Thr, [^{35}S]Met, [^{14}C]Leu, [^{14}C]Ala, [^{14}C]Ser, and [^{35}S]-Cys (Figure 1). However, no aminoacylation was observed with [^{14}C]Orn (Figure 1), [^{35}S]Hcy (Figures 1 and 3), [^{14}C]-Ile (Figure 2), or with [^{14}C]Asp and [^{14}C]Pro (not shown).

Table 1: Editing and tRNA^{Lys} Aminoacylations Catalyzed by LysRS[a]

		tRNA^{Lys} aminoacylation	
amino acid	editing k_{cat}/K_M (M^{-1} s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})	extent (%)
lysine	−	450000 ± 53000	100 ± 3
ornithine	580 ± 30	<0.3	<1
arginine	−	285 ± 14	90 ± 5
threonine	6 ± 1	28 ± 0.2	74 ± 3
methionine	4 ± 1	14 ± 1.3	58 ± 4
homocysteine	26 ± 2	<0.3	<1
cysteine	6 ± 1	0.8 ± 0.2	6 ± 2
leucine	4 ± 1	3.4 ± 0.5	20 ± 2
alanine	3 ± 1	1.7 ± 0.1	33 ± 5
serine	−	0.6 ± 0.1	10 ± 2

[a] For the noncognate amino acids, the k_{cat}/K_M values were calculated from rate measurements, V, at substrate concentrations $S < K_M$, according to $k_{cat}/K_M = V/E{\cdot}S$ where E is the concentration of LysRS. The rates of editing were determined from the amino acid-dependent AMP formation measured in the presence of 10−50 mM noncognate amino acid. The rates of ornithine editing were determined from ornithine lactam formation measured with 0.05−1.0 mM [^{14}C]ornithine. The rates of tRNA^{Lys} aminoacylation were measured with 0.0025−0.25 mM [^{14}C]amino acid. For the aminoacylation with lysine, K_M and k_{cat} values were 2.7 μM and 6.1 s^{-1}, respectively. Nonsaturating kinetics were observed in aminoacylation and editing reactions with noncognate amino acids. The shown extents of tRNA^{Lys} aminoacylation were obtained with 0.25 mM [^{14}C]amino acid and 0.4 μM LysRS. Lower extents of misaminoacylation were observed at lower concentrations of [^{14}C]amino acid and LysRS.

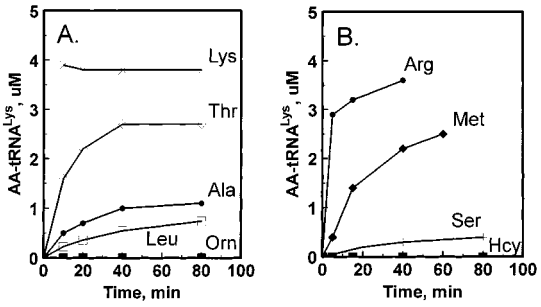


FIGURE 1: Time courses of tRNA^{Lys} aminoacylations catalyzed by LysRS. Reaction mixtures contained 4 μM tRNA^{Lys}, 0.4 μM LysRS, 2 mM ATP, and 0.25 mM radiolabeled amino acid in the standard buffer. Incorporation of the following radiolabeled amino acids into tRNA^{Lys} was determined by TCA precipitation. Panel A: lysine (×), threonine (◇), alanine (●), leucine (□), and ornithine (■). Panel B: arginine (●), methionine (◆), serine (+), and homocysteine (■). Small, not affected by incubation time, backgrounds of TCA-precipitable radioactivity, obtained in the absence of LysRS or tRNA, were subtracted from the results. No incorporation of the radiolabeled noncognate amino acids into tRNA was observed when 2 mM lysine was included in the reaction mixtures. No incorporation of the radiolabeled noncognate amino acids into TCA-precipitable material was observed in the absence of tRNA.

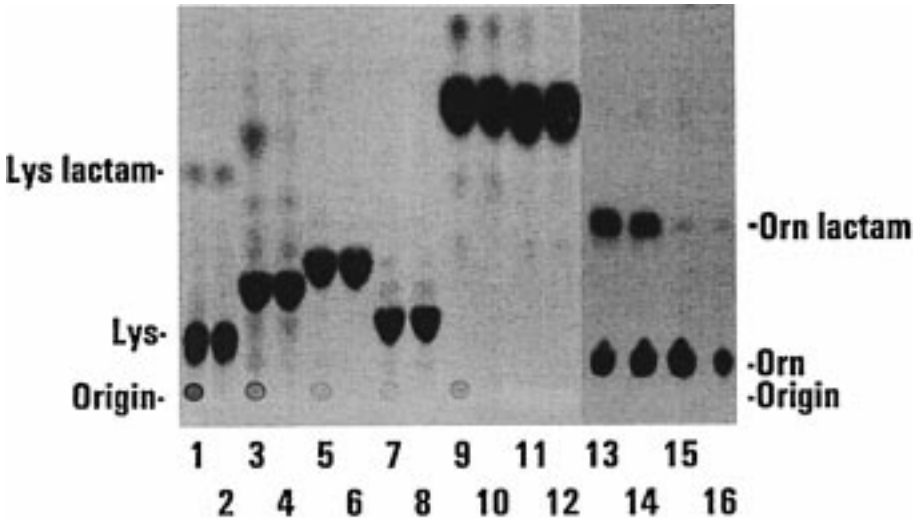


FIGURE 2: TLC analysis of $tRNA^{Lys}$ aminoacylation mixtures. Reaction mixtures contained 0.4 μM LysRS (lanes 1−14), 2 mM ATP, 0.25 mM [^{14}C]amino acid, and either 4 μM $tRNA^{Lys}$ (lanes 1, 3, 5, 7, 9, 11, and 13) or no tRNA (lanes 2, 4, 6, 8, 10, 12, and 14) in the standard buffer. Reaction mixtures containing [^{14}C]ornithine, ATP, and 0.4 μM ArgRS in the presence (lane 15) or absence of 10 μM $tRNA^{Arg}$ (lane 16) were also prepared. After 1 h at 37 °C, aliquots were subjected to TLC. An autoradiogram of TLC separations is shown. Reaction mixtures containing the following [^{14}C]amino acids were analyzed: lysine (lanes 1, 2), threonine (lanes 3, 4), alanine (lanes 5, 6), serine (lanes 7, 8), leucine (lanes 9, 10), isoleucine (lanes 11, 12), and ornithine (lanes 13−16). Positions of ornithine, lysine, and their lactams are indicated. Aminoacyl-$tRNA^{Lys}$ stays at the origin (lanes 1, 3, 5, 7, and 9). A major spot seen in each lane represents an unreacted amino acid. The identities of the fast moving spots in lanes 3 and 9 are not known.

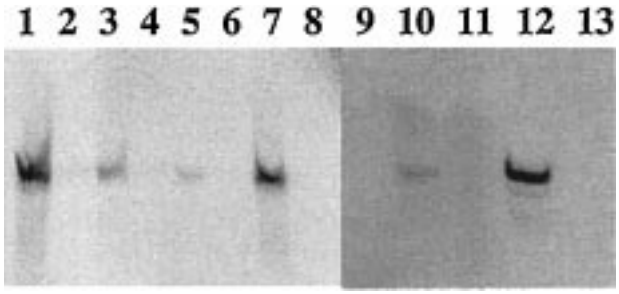


FIGURE 3: Detection of aminoacylated $tRNA^{Lys}$ by acid gel electrophoresis. Samples (3 μL) of aminoacylation mixtures described in the legend to Figure 1 were subjected to electrophoresis on 13% polyacrylamide gels in 0.1 M sodium acetate buffer pH 5.5 (12). An autoradiograph of a gel is shown. Aminoacylation mixtures containing the following radiolabeled amino acids in the presence (lanes 1, 3, 5, 7, 9, 10, 12) and absence of LysRS (lanes 2, 4, 6, 8, 11, and 13) were analyzed: [^{14}C]lysine (lanes 1, 2); [^{14}C]threonine (lanes 3, 4); [^{14}C]alanine (lanes 5, 6); [^{14}C]arginine (lanes 7, 8); [^{35}S]homocysteine (lane 9); [^{35}S]cysteine (lanes 10, 11); or [^{35}S]methionine (lanes 12, 13). Where present, radiolabeled bands comigrated with the $tRNA^{Lys}$ band (visualized under UV).

The incorporation of noncognate radiolabeled amino acids into $tRNA^{Lys}$ was abolished when lysine (2 mM) was included in the aminoacylation mixtures (not shown). When complete aminoacylation mixtures with [^{14}C]Arg, [^{14}C]Thr, [^{14}C]Ala, [^{35}S]Met, or [^{35}S]Cys (Figure 3) were subjected to PAGE on 13% gels at pH 5.5, a radiolabeled band associated with $tRNA^{Lys}$ was detected in each case. In addition, misacylated tRNA was quantitatively recovered from aminoacylation mixtures after phenol extraction (see Materials and Methods). Control experiments showed that no incorporation of radiolabeled amino acids into TCA-precipitable material occurred in the absence of $tRNA^{Lys}$ (Figure 1). These observations indicate that (i) LysRS is responsible for the formation of misacylated $tRNA^{Lys}$ and (ii) the misincorporation is not due to possible labeling of the enzyme by radiolabeled noncognate aminoacyl adenylates (*14*).

In misacylations of $tRNA^{Lys}$ with 0.25 mM radiolabeled amino acids (Figure 1), from 0.2 to 1.5% of the total radioactivity was incorporated into $tRNA^{Lys}$; in other experiments with lower concentrations of [^{14}C]Arg (0.03 mM), up to 10% of the radiolabeled amino acid can be incorporated into $tRNA^{Lys}$ (not shown). These degrees of incorporation are not due to contamination of the radiolabeled amino acids with lysine. In fact, TLC analyses showed that essentially no lysine was present in the preparations of the radiolabeled amino acids used in this work. For example, there was no radioactive material at lysine positions in lanes 4, 6, and 10 of Figure 2, showing TLC analyses of tRNA aminoacylation mixtures containing threonine, alanine, and leucine, respectively. In addition, kinetics of misacylations exclude the presence of lysine as a contaminant. If present, all contaminating lysine would be incorporated into tRNA within <1 min and no further increase in TCA-precipitable radioactivity would occur, which is not what was observed. Instead, a continuous increase in the TCA-precipitable material was observed up to 40−80 min (Figure 1). Finally, several misacylated $tRNA^{Lys}$ were isolated, and each was shown to yield a noncognate amino acid upon deacylation (see below).

Up to 500-fold variation in catalytic efficiencies (k_{cat}/K_M) was observed between noncognate amino acids (Table 1). Arginine was the most effcient noncognate amino acid substrate for $tRNA^{Lys}$ aminoacyaltion: its catalytic effciency was 1600-fold lower than the catalytic efficiency for the cognate lysine. Catalytic efficiencies for misacylations with threonine, methionine, leucine, alanine, serine, and cysteine were 16 000-, 32 000-, 132 000-, 264 000-, 562 000-, and 750 000-fold lower than the catalytic effciency for the aminoacylation with lysine. Although the misacylation reactions are slow in comparison to the aminoacylation with lysine, substantial levels (up to 90%) of $tRNA^{Lys}$ misacylation were observed (Figure 1 and Table 1). Such high levels of misacylation are possible because LysRS does not deacylate misacylated $tRNA^{Lys}$ (see below).

Table 2: Deacylation of Aminoacyl-tRNA^{Lys} in the Presence and Absence of LysRS[a]

	k (min^{-1})		
	+LysRS		
misacylated tRNA^{Lys}	−DTT	+DTT	−LysRS
2.2 μM [^{14}C]Lys-tRNA^{Lys}	0.014	0.53	0.017
1.4 μM [^{14}C]Arg-tRNA^{Lys}	0.040	0.040	0.046
0.76 μM [^{14}C]Thr-tRNA^{Lys}	0.046	0.046	0.014
0.16 μM [^{35}S]Met-tRNA^{Lys}	0.034	0.034	0.017

[a] Reactions were carried out at 37 °C in mixtures containing 0.16−2.2 μM radiolabeled AA-tRNA^{Lys} and 2 μM LysRS, in the presence and absence of 50 mM DTT. Nonenzymatic deacylation of AA-tRNA^{Lys} was not affected by DTT. First-order rate constants for deacylation, k, were calculated from measured deacylation half-lives, $t_{0.5}$, according to $k = \ln 2/t_{0.5}$. Individual measurements, repeated at least twice, agreed within ±20%.

Deacylation of AA-tRNA^{Lys}. Deacylation of misacylated tRNA^{Lys} was studied for two reasons: (i) to confirm the identity of the amino acid attached to tRNA^{Lys}; (ii) to determine whether LysRS possesses a post-transfer editing mechanism. Because deacylation of Lys-tRNA^{Lys} by LysRS requires a thiol as an acceptor for lysine (*8*), deacylations were carried out in the presence and absence of DTT.

When reactions were carried out in the absence of DTT, rate constants for slow spontaneous deacylation of [^{14}C]Lys-tRNA^{Lys} and [^{14}C]Arg-tRNA^{Lys} were not significantly affected by the presence of LysRS (Table 2). Deacylations of [^{14}C]Thr-tRNA^{Lys} and [^{35}S]Met-tRNA^{Lys} were only 2−3-fold faster in the presence of LysRS that in its absence. Thus, LysRS essentially does not possess a post-transfer editing mechanism.

When reactions were carried out in the presence of DTT, [^{14}C]Lys -tRNA^{Lys} was deacylated about 30-fold faster in the presence of LysRS than in its absence (Table 2), as expected (*8*). TLC analysis confirmed that a thioester, Lys-DTT (*8*), was a product of this deacylation reaction. DTT-dependent enzymatic deacylation of [^{14}C]Lys-tRNA^{Lys} was abolished by the presence of 2 mM lysine which prevents binding of the [^{14}C]Lys moiety in [^{14}C]Lys-tRNA^{Lys} to LysRS (*8*). However, DTT did not affect deacylations of [^{14}C]Arg-tRNA^{Lys}, [^{14}C]Thr-tRNA^{Lys}, and [^{35}S]Met-tRNA^{Lys} in the presence or absence of LysRS. Corresponding free noncognate amino acids were detected as products of slow deacylations of [^{14}C]Arg-tRNA^{Lys}, [^{14}C]Thr-tRNA^{Lys}, and [^{35}S]Met-tRNA^{Lys} in the presence or absence of DTT. Because [^{14}C]Arg-tRNA^{Lys}, [^{14}C]Thr-tRNA^{Lys}, and [^{35}S]Met-tRNA^{Lys}, in contrast to [^{14}C]Lys -tRNA^{Lys}, are not substrates for the thiolysis reaction catalyzed by LysRS, it appears that the noncognate amino acid moieties in the misacylated tRNAs are not bound by the enzyme.

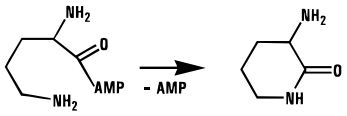
Editing of Ornithine Occurs by Cyclization to the Lactam. In view of the relatively relaxed amino acid specificity of LysRS in the tRNA aminoacylation reaction, it was surprising that ornithine failed to be transferred to tRNA^{Lys}. An underlying reason for this could be the existence of an efficient editing mechanism that prevents misacylation of tRNA^{Lys} with ornithine. From our knowledge of intramolecularly catalyzed reactions, it is expected that such an editing mechanism would yield ornithine lactam by the facile cyclization of ornithyl adenylate. TLC analyses of tRNA^{Lys} aminoacylation mixtures containing [^{14}C]Orn revealed that a new ^{14}C-labeled product indeed formed (lanes 13 and 14 in Figure 2). The product co-chromatographed with an authentic ornithine lactam standard in several chromatographic systems and was resistant to NaOH treatment (not shown). These properties are consistent with its identity as ornithine lactam. Formation of the lactam required ATP and LysRS, and was abolished by the presence of 2 mM lysine (Table 3). Arginine and histidine did not prevent formation of the lactam. tRNA^{Lys} increased the rate of ornithine lactam formation by 70% (Table 3). It was also determined that ArgRS did not catalyze conversion of ornithine to the lactam nor misacylation of tRNA^{Arg} with ornithine (lanes 15 and 16 in Figure 2).

Table 3: Requirements for the Synthesis of Ornithine Lactam[a]

reaction mixture	rate of ornithine lactam formation (%)
complete	100 ± 3 (1.2 μM/min)
−LysRS	<1
−ATP	<1
+tRNA^{Lys}, 8 μM	170 ± 10
+Lys, 2 mM	<1
+Arg, 2 mM	56 ± 7
+His, 2 mM	28 ± 2
−PPase	72 ± 9

[a] Reactions were carried out at 37 °C in mixtures containing 0.22 mM [^{14}C]ornithine, 2 mM ATP, and 0.4 μM LysRS in the standard buffer. The lactam was separated from ornithine by TLC.

Scheme 1



The characteristics of the conversion of ornithine to the lactam indicate that ornithine is first misactivated by LysRS to form Orn-AMP. The side chain of ornithine then attacks the activated carboxyl group of Orn-AMP, displacing AMP and giving ornithine lactam (Scheme 1).

As can be seen in lanes 1 and 2 of Figure 2, a small fraction of lysine in the aminoacylation mixtures was converted to lysine lactam. The conversion to lysine lactam occurred in the absence or presence of tRNA with $k_{cat} = 0.00065$ s^{-1}, >230-fold slower than the conversion of ornithine to ornithine lactam ($k = 0.15$ s^{-1} at 1 mM ornithine).

Stoichiometry of ATP Hydrolysis and Ornithine Lactam Formation. It is unclear whether formation of ornithine lactam is a major editing pathway or some of Orn-AMP is also hydrolyzed to ornithine and AMP. To determine the contribution of the two possible pathways of Orn-AMP rejection to overall editing of ornithine by LysRS, measurements of AMP and ornithine lactam were carried out. As shown in Table 4, the average ratios of AMP/lactam were 1.14 and 1.23 in the absence and presence of tRNA^{Lys}, respectively. Thus, conversion of ornithine to the lactam is a major editing pathway, contributing about 80% to the overall editing of ornithine.

Identification of ornithine lactam establishes that ornithine is edited by LysRS. A failure to detect editing of ornithine by LysRS in previous work (*8*) can be traced to the use of high (50 mM) concentrations of nonradiolabeled ornithine which apparently inhibited the enzyme. In the present work, ornithine lactam was detected with 0.05−2.5 mM [^{14}C]-ornithine.

Table 4: Relationship between Hydrolysis of ATP to AMP and the Conversion of Ornithine to the Lactam Catalyzed by LysRS[a]

time (min)	AMP (μM)		ornithine lactam (μM)		AMP/ornithine lactam	
	−tRNA	+tRNA	−tRNA	+tRNA	−tRNA	+tRNA
10	19.5	29	17	23	1.14	1.26
20	36	49	31	40	1.16	1.23
30	58	68	51	54	1.13	1.26
40	82	97	71	82	1.15	1.18

[a] Reactions were carried out at 37 °C in mixtures containing 2.5 mM [^{14}C]ornithine, 2.5 mM [α-^{32}P]ATP (100 Ci/mol), and 0.4 μM LysRS in standard buffer. Where indicated, 5 μM tRNA^{Lys} was present.

DISCUSSION

This work demonstrates the following: (i) *E. coli* LysRS, a class II enzyme, catalyzes misacylations of tRNA^{Lys} with arginine, threonine, methionine, leucine, alanine, serine, and cysteine. (ii) LysRS does not possess a post-transfer editing mechanism. (iii) LysRS edits ornithine by converting it into ornithine lactam by a pre-transfer mechanism.

Of the AARSs studied so far (*1*−*3*), LysRS is the only example of an enzyme that relatively efficiently misacylates cognate tRNA with noncognate amino acids. Among several examples of misacylations catalyzed by LysRS, formation of Arg-tRNA^{Lys} was the most efficient: the catalytic efficiency was 1600-fold lower than that observed for the correct aminoacylation with lysine. This misacylation error rate is higher than most error rates observed in translation in vivo (*2*). It remains to be determined whether Arg-tRNA^{Lys} formation occurs in vivo and, if not, how it is prevented. Because LysRS lacks a post-transfer editing mechanism, up to 90% tRNA^{Lys} can be misaminoacylated in vitro. A practical aspect of the present work is that it provides a means of preparing misacylated tRNA substrates for biological synthesis of artificial peptides with a variety of amino acids inserted at lysine codons.

Although *E. coli* LysRS was purified in 1967 (*15*) and its crystallographic structure determined in 1995 (*16*), the amino acid selectivity of the enzyme has only recently begun to be examined (*8*). A related enzyme, yeast LysRS, does not misacylate tRNA with noncognate amino acids (*17*); misacylation rates $\geq$0.1% of the tRNA aminoacylation with lysine would have been detected in that study. However, in another study (*18*), yeast LysRS has been claimed to misacylate yeast tRNA^{Lys} with all 20 protein amino acids: catalytic efficiencies were anywhere from 0.1 to 2.5% of that observed with lysine, but the levels of tRNA^{Lys} misacylation were not reported and the discrepancies with the original study of yeast LysRS (*17*) were not addressed. A peculiar aspect of the misacylations reported in (*18*) is that K_m values for most noncognate amino acids are similar to, or lower than, the K_m value for the cognate lysine. One possible reason for artificially low K_m values would be contamination of noncognate amino acids by traces of lysine.

Although, as described in the present study, most of the noncognate amino acids that were transferred to tRNA^{Lys} were also edited, the catalytic efficiencies for editing reactions were lower than, or comparable to, catalytic efficiencies of the corresponding misacylation reactions (Table 1). In addition, LysRS did not exhibit hydrolytic activity toward misacylated tRNA^{Lys}. Thus, relatively inefficient pre-transfer editing and the absence of a post-transfer editing mechanism account for the relaxed amino acid selectivity of tRNA^{Lys} aminoacylation.

However, despite relatively relaxed amino acid selectivity toward some noncognate amino acids, LysRS exhibits absolute selectivity against Hcy and Orn in tRNA^{Lys} aminoacylation reaction. Hcy and Orn are precluded from being attached to tRNA^{Lys} by the existence of specific editing mechanisms. Since lysine and ornithine are structurally very similar, it may not be surprising that LysRS evolved an editing mechanism directed against ornithine. This has been well documented for other closely related pairs of amino acids, such as isoleucine/valine (*4*, *7*, *10*, *19*, *20*), isoleucine/homocysteine (*5*, *10*, *20*), isoleucine/cysteine (*5*, *10*, *20*), valine/threonine (*6*, *19*, *21*), valine/cysteine (*5*, *6*, *10*, *20*), methionine/homocysteine (*5*, *22*), alanine/glycine and alanine/serine (*23*), phenylalanine/tyrosine (*24*), and leucine/homocysteine (*25*). As shown here, Orn is converted into the lactam by a pre-transfer editing mechanism, similar to the mechanism of Hcy conversion into thiolactone by MetRS (*5*), IleRS (*5*), LeuRS (*25*), and LysRS (*8*), as well as to the mechanism of homoserine conversion to lactone by LysRS (*8*), IleRS, and ValRS (*2*). Among several noncognate amino acids that are edited by LysRS, ornithine exhibits the highest catalytic efficiency of editing, equal to 0.1% of the catalytic efficiency of Lys-tRNA^{Lys} formation. This means that the error rate for misactivation of ornithine is relatively high and that the editing mechanism of LysRS is most likely directed against ornithine in vivo.

Accumulation of ornithine lactam (*13*, *26*), in addition to ornithine, is associated with two human genetic disorders: gyrate atrophy of the choroid and retina, with symptoms limited to the eye; and the hyperornithinemia−hyperammonemia−homocytrullinemia (HHH) syndrome with a variety of symptoms occurring during the newborn period or delayed until late adulthood. It is not known how ornithine lactam forms in the human body. Conversion of ornithine to the lactam as a result of an error-editing function of LysRS is one plausible mechanism.

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