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Amino Acid Toxicities of *Escherichia coli* That Are Prevented by Leucyl-tRNA Synthetase Amino Acid Editing▽

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Leucyl-tRNA synthetase (LeuRS) has evolved an editing function to clear misactivated amino acids. An *Escherichia coli*-based assay was established to identify amino acids that compromise the fidelity of LeuRS and translation. Multiple nonstandard as well as standard amino acids were toxic to the cell when LeuRS editing was inactivated.

In the first step of protein synthesis, an aminoacyl-tRNA synthetase (aaRSs) is responsible for linking a single standard amino acid to its correct set of tRNA isoacceptors (11, 20). About half of the family of 20 aaRSs are challenged to distinguish among closely related amino acids and have evolved amino-acid-editing mechanisms to clear their mistakes (10). These editing mechanisms maintain translational fidelity by impeding the production of "statistical proteins," which is hazardous to cell viability.

The fidelity of most aaRSs that edit is threatened by a limited number of noncognate standard amino acids. For example, isoleucyl-tRNA synthetase (IleRS) must distinguish between isoleucine and valine, which differ by a single missing methyl group (2, 5, 6, 9, 22, 23). Valyl-tRNA synthetase (ValRS) editing targets threonine, which has a hydroxyl group that is isosteric to the methyl moiety in valine (7, 8). Previously, we determined that leucyl-tRNA synthetase (LeuRS), which is homologous to IleRS and ValRS, misactivates a wide array of amino acids in vitro (19). Thus, we hypothesized that numerous structurally diverse amino acids might compete effectively for binding in the larger leucine-binding pocket of LeuRS (17) for aminoacylation to $tRNA^{Leu}$.

We sought to determine which of the amino acids that were misactivated by LeuRS might be detrimental to the cell if the editing activity of LeuRS was dysfunctional. This would also provide insight into which amino acids were actually targeted by the LeuRS editing activity in vivo to maintain the fidelity of translation. We employed the editing-defective TT/VV LeuRS mutant strain of *Escherichia coli* (26) to investigate the intracellular toxicity of LeuRS-misactivated amino acids. This mutated LeuRS has two conserved threonines that have been replaced by valines in the editing-active site (26). Aminoacylation is unaffected, but hydrolytic editing activity is abolished to stably produce mischarged Ile-$tRNA^{Leu}$ in vitro.

We cotransformed the *E. coli* strain KL231, which has a temperature-sensitive LeuRS mutation (18) with plasmid pGP1-2, which carries the gene for T7 RNA polymerase (25), and plasmid pYZHAI3, which expresses the editing-defective TT/VV LeuRS mutant (26). Transformants were selected as described previously (12, 18). The editing-defective TT/VV LeuRS mutant enzyme as well as a wild-type LeuRS control (p15ec3-1 [19]) complemented the temperature-sensitive strain at 42°C (Fig. 1). In vitro aminoacylation assays indicated that mischarging by the TT/VV LeuRS mutant was slightly increased at 42°C compared to that at 30°C (data not shown), which could increase cell sensitivity to the editing defect at the higher temperature.

Previously, we showed that excess isoleucine was inhibitory to the growth of *E. coli* bacteria that were dependent on an editing-defective LeuRS (12). Herein, we expanded our toxicity studies to include all of the standard amino acids to identify those amino acids that threaten LeuRS fidelity in vivo. A zone of growth inhibition, or a halo, around a central well that contained concentrated nonleucine amino acids showed that among the standard amino acids, isoleucine, valine, and methionine were toxic to *E. coli* cells that were dependent on the editing-defective TT/VV LeuRS mutant for aminoacylation (Fig. 1A). We also tested a series of nonstandard amino acids that accumulate in metabolic pathways or act as signaling molecules within bacteria (3, 13–15, 24). Norvaline, norleucine, homoserine, and homocysteine yielded significant halos (Fig. 1B). In contrast, cells that were complemented by the wild-type LeuRS failed to produce a halo in the presence of high concentrations (near the limits of saturation) of any of these nonleucine amino acids. This demonstrates that the robust post-transfer editing function of LeuRS protects the cell from potential amino acid toxicities.

We also investigated the cell growth of *E. coli* KL231 cells harboring either the wild type or the editing-defective LeuRS TT/VV mutation in liquid minimal medium cultures. When the LeuRS TT/VV mutation was present, excess methionine or valine slowed growth rates in comparison to that of the cells complemented by the wild-type LeuRS (Fig. 2). In addition, these cells reached a lower plateau, indicating that growth was

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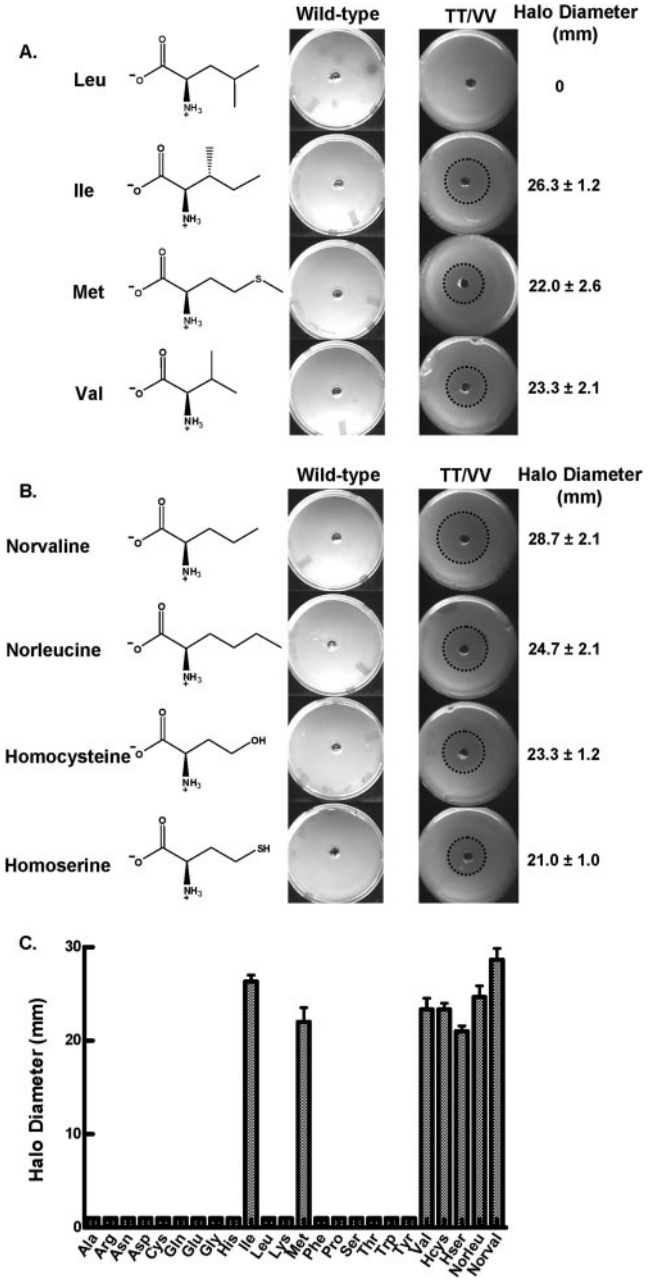

FIG. 1. Amino acid toxicities to *E. coli* KL231 cells that were dependent on the editing-defective LeuRS TT/VV mutation. Halo assays were carried out as described previously (12). (A) Standard aliphatic amino acids. (B) Nonstandard aliphatic amino acids. (C) Histogram representing the halo diameters for all tested amino acids. Zones of inhibition or halos are marked by dashed circles. Standard deviations were based on the assay repeated at least in triplicate.

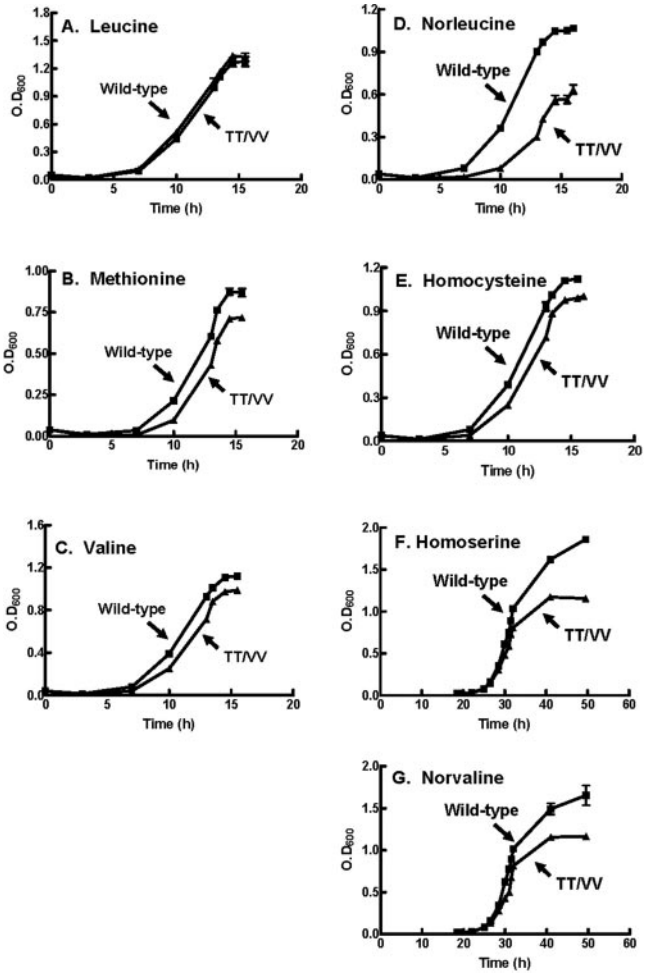

FIG. 2. Growth curves *E. coli* KL231 in the presence of toxic levels of nonleucine amino acids. Transformed *E. coli* KL231 was prepared, and its growth rates were measured as previously described (12). *E. coli* KL231 harboring wild-type (■) or the TT/VV editing-defective mutant (▲) LeuRS was grown in liquid minimal medium that contained 16 mM of the following amino acids: (A) leucine, (B) methionine, (C) valine, (D) norleucine, (E) homocysteine, (F) homoserine, and (G) norvaline. Error bars are based on assays that were repeated in triplicate and are present for each point.

stunted, presumably as statistical protein mutations accumulated within the cell due to the LeuRS editing defect (Fig. 2). Likewise, liquid growth cultures showed that the nonstandard amino acids norleucine, norvaline, homocysteine, and homoserine reduced growth rates and lowered plateaus when *E. coli* was dependent on the editing-defective LeuRS (Fig. 2). These changes in growth patterns varied for different amino acids and between experiments due to the statistical nature of the accumulation of errors during protein synthesis.

We carried out a dose-dependent analysis of the amino acids that were toxic to *E. coli* KL231 in the presence of an editing-defective LeuRS (Fig. 3). We also determined the 50% inhibitory concentration (IC_{50}) values (± standard deviations) for the amino acids to quantitate their toxicities, as follows: 27.0 ± 4.7 mM for isoleucine, 36.6 ± 4.2 mM for methionine, 46.3 ± 25 mM for valine, and 14.1 ± 5.1 mM for homoserine. The unbranched, aliphatic norvaline and norleucine exhibited the most potent toxicities, with IC_{50} values of 4.2 ± 2.8 mM and 8.2 ± 0.5 mM, respectively. By comparison, the standard amino acid isoleucine had an IC_{50} value of 27.0 ± 4.7 mM. Despite repeated attempts, a reproducible IC_{50} value for homocysteine could not be measured because of heterogeneity in the sample and low solubility. Likewise, the IC_{50} value for valine varied significantly

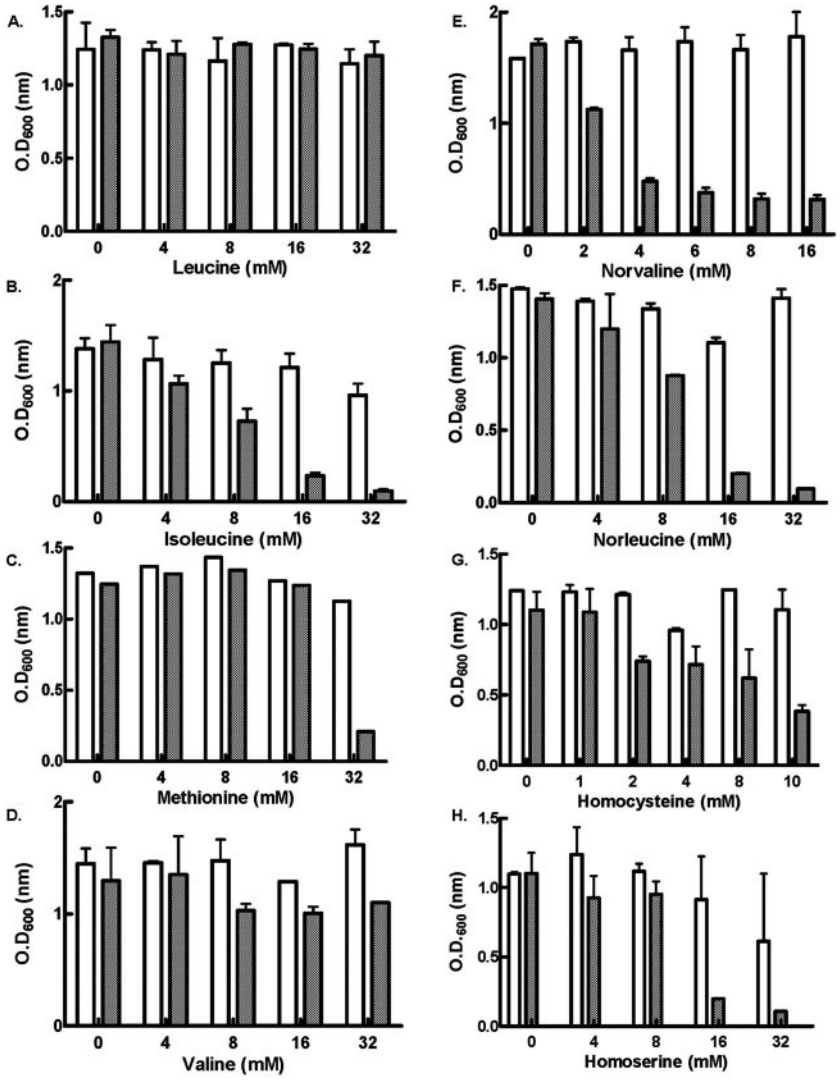

FIG. 3. Amino acid dose-dependent viability decrease of *E. coli* KL231 that is dependent on the wild type or the editing-defective TT/VV mutant LeuRS. *E. coli* KL231 harboring wild-type (open bars) or the TT/VV editing-defective mutant (solid bars) LeuRS was grown in liquid minimal medium containing increasing concentrations as indicated for the following amino acids: (A) leucine, (B) isoleucine, (C) methionine, (D) valine, (E) norvaline, (F) norleucine, (G) homocysteine, and (H) homoserine. Optical densities at 600 nm (OD_{600}) were measured in triplicate after 24 h of growth to obtain standard deviations.

in repeated experiments because its IC_{50} value was high and approached the limits of solubility under the conditions used. In addition, potential IC_{50} values for effects on the wild-type enzyme could not be measured because of the relatively low solubility of each of these aliphatic amino acids.

Overall, these combined results suggest that nonstandard amino acids might be a greater threat to the LeuRS-dependent fidelity of protein synthesis than standard amino acids. In the case of norvaline, it has been shown that intracellular levels of norvaline are low compared to those of leucine but can be significantly increased under conditions that induce high expression of recombinant proteins in *E. coli* (1). Interestingly, norvaline has been shown to at least partially bypass the LeuRS editing mechanism and to substitute for leucine under cell growth conditions that require high expression of recombinant proteins in *E. coli* (1). However, our results emphasize that norvaline is subject to LeuRS amino acid editing at levels that control its toxicity.

Our investigation supports the possibility that LeuRS has the complicated challenge of discriminating between multiple standard and nonstandard amino acids in vivo. As with other aaRSs (4, 21), LeuRS is required to block amino acid toxicity to the cell with high translational fidelity. While some aaRSs can achieve this level of discrimination within a single aminoacylation-active site to maintain accurate protein synthesis, LeuRS and other aaRSs have acquired a second active hydrolytic site to edit misactivated amino acids (10). Inactivation of the LeuRS editing activity clearly hinders cell growth. This is likely due to a steady accumulation of errors during translation that would yield misfolded and/or inactivated proteins (16). As these statistically

generated protein mutations accumulate, intracellular processes would be compromised to lower cell viability and survival. These high fidelity requirements of LeuRS for protein synthesis are consistent with the early acquisition of its editing domain during evolution (27).

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