Isoleucyl-tRNA synthetase editing domain accepts broad range of amino acids that are efficiently discriminated at the synthetic active site

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Aminoacyl-tRNA synthetases (aaRSs) activate amino acids and transfer them to cognate tRNAs. Some aaRSs cannot establish a required specificity in amino acid recognition and thus may erroneously activate noncognate amino acids with a frequency higher than 10^{-3}. These enzymes evolved a separate editing domain to hydrolyze formed misaminoacylated tRNAs (post-transfer editing). An initial model of discrimination at the editing domain proposed that binding of the cognate amino acid is prevented by a steric clash. Yet, we showed that the cognate amino acid may bind at the editing site, but unproductively (previously published in Dulic et al. J Mol Biol (2018) 430, 1-16). To understand better what shaped selectivity of the editing domain we have used isoleucyl-tRNA synthetase (IleRS) as a model enzyme in our recent (previously published in Bilus et al. J Mol Biol (2019) 431, 1284-1297; Zivkovic et al. FEBS J (2019) early view) and novel work. We tested a broad range of substrates belonging to i) proteinogenic (Ile, Ala, Val, Leu, Thr, Ser and Met), ii) nonproteinogenic (α-aminobutyrate, norvaline (Nva) and norleucine), and iii) synthetic (di- and tri-γ-fluoro-α-aminobutyrate) amino acids. Among them, only Val and Nva mimic well the cognate Ile and were poorly discriminated (< 200-fold), while the others were well discriminated at the IleRS synthetic site (500- to 10^6-fold). Nevertheless, we prepared misacylated tRNAs with all tested amino acids and followed their hydrolysis in an independent assay. Surprisingly, all misacylated tRNAs were hydrolyzed by IleRS at similar rates (35-70 s^{-1}). Thus, how efficient amino acids were discriminated at the synthetic site and consequently whether these amino acids posed an evolutionary threat to translation fidelity does not determine the efficiency of their post-transfer editing. Only the cognate Ile-$tRNA^{Ile}$ was hydrolyzed slowly (0.058 s^{-1}), suggesting that this is the main requirement that shaped specificity of the editing domain.