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Title: Characterization of the Alanyl-tRNA Synthetase Fidelity Mechanism by Kinetic Analysis of Editing Domain Mutants

Aminoacyl-tRNA synthetases(ARS's) are enzymes responsible for joining amino acids with their cognate tRNA's before they can be incorporated into protein synthesis in a process called aminoacylation. They help to ensure the fidelity of the process in several ways. Alanyl-tRNA Synthetase (AlaRS) limits the availability of its catalytic domain to primarily alanine by steric exclusion, but serine and glycine are occasionally misincorporated because of their small size. AlaRS limits the production of Gly-, and Ser-$tRNA^{ala}$ by using a separate editing domain to deacylate these products by hydrolysis. To gain insight into the function of the AlaRS editing domain, and to develop a kinetic mechanism for a mouse model of a progressive neurodegenerative disorder the causative mutation A734E (*E.Coli* I677E) was studied in *E.Coli* AlaRS. Kinetic analysis of I677E using Ser-$tRNA^{ala}$ indicates that this mutant is significantly less active in deacylation than wild-type AlaRS. The K_M and k_{cat} for editing could not be determined because it lacks saturation behavior; however, the second order rate constant k_{cat}/K_M was found to be $1.0 \times 10^5\ M^{-1}\ s^{-1}$. This is 6.6 fold lower than the value for wild-type AlaRS of $6.6 \times 10^5\ M^{-1}\ s^{-1}$. Substrate specificity of the mutant was verified in experiments using Ala-$tRNA^{ala}$. These indicate slower rates of hydrolysis compared to those observed with Ser-$tRNA^{ala}$, and a k_{cat}/K_M value similar to wild-type. Aminoacylation experiments were also performed and these indicate that the I677E mutation's effect is limited to the editing domain as the enzyme retains

similar activity to wild-type. The results of this study are significant because they provide the first quantitative characterization of the I677E mutation.