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Kinetic Analysis on *Escherichia coli* Enzyme Equivalent of Human Histidyl-tRNA Synthetase Mutation Associated with Usher Syndrome Type IIIB

The twenty enzymes known as aminoacyl-tRNA synthetases (ARSs) are crucial components in protein synthesis. Specifically, each ARS is responsible for catalyzing the two-step reaction where a specific amino acid is attached to its specific transfer ribonucleic acid (tRNA). Histidyl-tRNA synthetase, which attaches histidine to its cognate $tRNA^{His}$, represents one of 20 such standard enzymes. A single point mutation in the human version of this enzyme (HARS), Y454S, was found in the Amish population, which causes the rare disorder Usher syndrome type IIIB (USH3B). This mutation is located within the interface region of the bound $tRNA^{His}$ and HARS complex.

This research studied the corresponding mutation (R375S) in the *E. coli* enzyme to analyze its function in a well-characterized system. It was hypothesized this R375S mutation would affect the binding specificity of the *E. coli* HisRS enzyme. It was predicted that, with wild type (wt)-$tRNA^{His}$ and amber (amb)-$tRNA^{His}$, the substitution in HisRS would cause both the Km and Vmax to decrease compared to the Wt enzyme. To determine this, polymerase chain reaction mutagenesis, protein purification, tRNA purification, active site titration assays, and aminoacylation assays were performed. From analysis of the data it was determined the specificity constants for aminoacylation of R375S HisRS with wt-$tRNA^{His}$ and amb-$tRNA^{His}$ were 13.7 M^{-1} s^{-1} and 8.96 M^{-1} s^{-1}, respectively. The specificity constants for aminoacylation of Wt HisRS with wt-$tRNA^{His}$ and amb-$tRNA^{His}$ were 12.4 $M^{-1}s^{-1}$ and 14.79 $M^{-1}s^{-1}$. None of these specificity constants were significantly different from one another, rejecting the hypothesis. This may be due to the USH3B mutation affecting a function of the histidyl-tRNA synthetase enzyme that evolved after *E. coli* bacteria. Future research will be required to determine if this is the case and if so, what function of the enzyme is being affected that causes the phenotypical symptoms of this syndrome.