On the efficiency of an enzyme that can incorporate non-natural amino acids into proteins

Jacquelyn Kelty
Indiana University - Purdue University Fort Wayne

Seja Culpepper
Indiana University - Purdue University Fort Wayne

Ryan Curtis
Indiana University - Purdue University Fort Wayne
On the efficiency of an enzyme that can incorporate non-natural amino acids into proteins
Jacquelyn Kelty, Seja Culpepper, Ryan Curtis, and Eric Tippmann
Department of Chemistry, Indiana University-Purdue University Fort Wayne

Introduction
Expanding the genetic code of an organism opens new avenues to modulate protein function. Interestingly, all known organisms use the same 20 common amino acids to make all the proteins on the planet—even though, genetically, we could sustain many more. Here, we altered an enzyme that naturally incorporated the common amino acid leucine into proteins. The alterations, or mutations, caused the enzyme to prefer a non-natural amino acid over its normal leucine partner. There have been more than 100 non-natural amino acids that have been successfully incorporated into proteins using this method, but the non-natural amino acid tested in this experiment is the only one that contains a metal. The focus of this project is to make the enzyme and test just how efficiently the enzyme incorporates the non-natural amino acid.

Methods and Results
The first goal was to obtain and purify the enzyme Leucyl amino acyl tRNA synthase (aaRS) found in E. coli. It was purified by using affinity chromatography. The desired enzyme binds to the 6xHis tag. Low concentrations of imidazole were run through to elute unwanted proteins and then a high concentration of imidazole is run through to collect the desired enzyme.

The second goal was to determine how efficiently the purified enzyme reacts with the previously made non-natural amino acid ferrocenyl cysteine. The assay needed to do so must be validated so a different natural enzyme with known activity is used to prove the assay. The enzyme used is Tyr RS found in M. Jannaschii.

The overall goal of this research is to see how effectively the non-natural amino acid ferrocenyl cysteine reacts with the enzyme leucyl amino acyl tRNA synthase. We want the enzyme to react efficiently with this non-natural amino acid even in the presence of its natural competitor leucine. By proving this interaction effective, we can insert this non-natural amino acid into proteins in order to alter their function. The ability to alter a protein’s function can provide advances in both the medical and biochemical fields.

Acknowledgements
We would like to thank the department of chemistry for allowing us space to do our research and also Dr. Tippmann for allowing us to work under him.

References