Investigating Catalytically Important in *Escherichia coli* Prolyl-tRNA Synthetase Using Site-Directed Mutagenesis and Computational Studies

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Abstract:

This research project is a continuing effort focused on the molecular-level understanding of the relationship between structure, dynamics, and function in aminoacyl-tRNA synthetases (AARSs). Recent computational studies have identified four charged amino acid residues at the active site [three positively charged arginine (R) and one negatively charged glutamic acid (E)] that are directly involved in catalysis in *Escherichia coli* Prolyl-tRNA Synthetase (Ec ProRS). Our current objective is to experimentally confirm these computational findings by mutating these residues to a neutral amino acid, alanine, as well as two of the amino acids (E111 and R450) to aspartic acid, a shorter negatively charged amino acid. These mutations are also being run computationally in order to compare results and validate our computational model used. By studying the kinetics and catalytic rate of these mutations, we can determine the importance of these specific active site residues, indicated to be directly involved in the enzymatic ProRS reaction. This knowledge can potentially be used to develop selective drug against pathogenic AARSs. AARSs are potential drug targets as they play a key role in protein synthesis in all living organisms. Herein, we will present the preliminary result of our work.

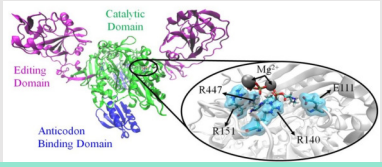
Background:

- **Aminoacyl-tRNA Synthetases**
  - Function: Help form the proteins required for life
    - Proteins are large, complex molecules consisting of amino acids
      - Ex. Kinesin, insulin, hemoglobin
  - Precise Function: unite an amino acid with the matching tRNA through aminoacylation

$$AA + AARS + ATP \rightleftharpoons AARS^{*}AA\text{-}AMP + PP_i$$

$$AARS^{*}AA\text{-}AMP + tRNA \rightleftharpoons AA\text{-}tRNA + AMP + AARS$$

- **Prolyl-tRNA Synthetases (ProRSs)**
- Catalyze the covalent attachment of proline to $tRNA^{Pro}$.
- *E. coli* ProRS possesses an editing mechanism and a separate domain to hydrolyze Ala-$tRNA^{Pro}$ [*1*].
- Certain residues have been found to have important roles in this catalysis:
  - E111, R140, R151, R447



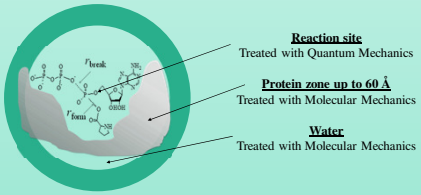
Resources: (1)Beuning et al. (2001) *J. Biol. Chem.* 276, 30779-307856) Schultz et al. (1961) *J. Gen. Physiol.* 44, 1189-1199
(2) Muino et al.(2009) *J. Biophys. B.*, 2572-2577
(3) Hixon, et al. (2009) *MDPI, 2: 1155-1176*
(4) Vivian et al (2001), *Biophys J.*, 80, 2039-2109.

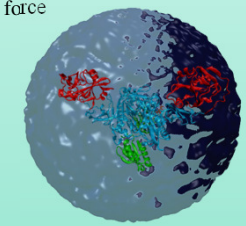
Objectives:

Confirm	Understand	Validate
Confirm the catalytic importance of arginine and glutamic acid residues from computational findings	Further understand how the structure of individual amino acids can effect protein structure, function, and dynamics	Validate our computational tools for studying protein dynamics

Computational Methods:

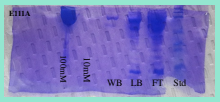
1. Use Protein Data Bank to access the 3D form of ProRS
2. Add substrates to the active site
3. Solvate structure in 60 A of water
4. Treat the system with QM/MM
5. Run umbrella sampling to form a potential mean force

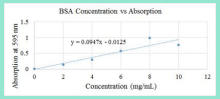


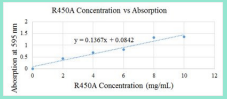


Experimental Methods:

1. Primer Design:
   Ex. Arginine CGT to Alanine GCT
2. Polymerase Chain Reaction (PCR): Amplifies mutant DNA
3. DPN1 Digestion: Removes Parent DNA
4. Transformation with XL1 Blue Cells: Competent cells used for their ability to pick up and amplify foreign plasmids
5. DNA Purification and Quantification
6. Sanger Sequencing: Done at UW- Madison
7. Transformation with SG13009 Cells: Competent cells used for their ability to pick up foreign plasmids and express proteins
8. Protein Overexpression: Using IPTG
9. Protein Prep/Purification: Cobalt Affinity Column with imidazole wash
10. Protein Quantification: Biorad assay:







Then the protein samples were sent to the research group of Dr. Musier-Forsyth at Ohio State University for kinetic assays.

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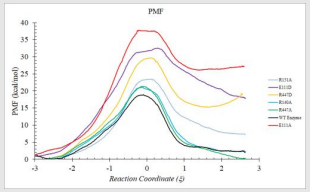
Results:

Computational:

- All mutations caused an increase in the activation barrier indicating decrease in the catalytic rate



Experimental:

❖ Rate of aminoacylation pmol/sec

ProRS	10nM	100nM	250nM
Wildtype	0.000185	0.0046	0.0039
E111A	-	0	-
E111D	0.0000983	0	0.0008
R140A	-	0.0008	-
R151A	-	0	-
R447A	-	0	-
R447D	-	0	-

Only E111D and R140A remain slightly active after mutation. This table verifies the catalytic importance of these residues.

Conclusion:

- R447 displays important electrostatic interactions with the phosphate group of ATP
  - Neutralization or alteration of the positive charge of this arginine has severe effect on catalysis
- E111 is important for proline binding
  - Neutralization or alteration of the charge caused an increase in the activation barrier and a decrease in thermodynamic favorability
- All other mutations rendered the enzyme inactive, validating their catalytic importance

Future Directions:

This study follows the catalysis of step one of the reaction. In the future, we hope to study the catalysis of the second step of the reaction, the binding of the tRNA.