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Characterization of an amino-acyl tRNA enzyme from M. jannaschii for genetic applications

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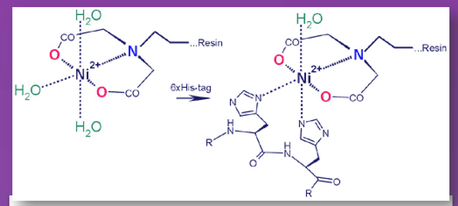
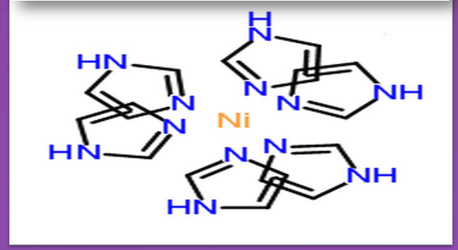
Background:

An amino acyl t-RNA enzyme is one the most important and ancient of all enzymes. All organisms have them and their "job" is to make sure that the correct amino acid is inserted in the correct order of every protein. While our DNA determines the order of amino acids and proteins, it is this class of enzymes that makes sure the DNA's code is followed with very few mistakes. Recently, these enzymes have been evolved away from their natural substrates so that they actually prefer non-natural amino acids



Scheme 1: His-tagged enzyme Tyr RS binding to Nickel



Scheme 2: Imidazole binding to Nickel.

Methods and Results:

The first goal is to obtain and purify the enzyme from bacterial expression cultures. The enzyme has a special affinity tag that facilitates its purification from the hundreds of other bacterial proteins. The second goal is to determine how efficiently the enzyme catalyzes the reaction with tyrosine. To that end, we performed affinity chromatography via Nickel resin (Scheme 1) during which the histidine tags of the enzyme bind to the nickel resin while impurities elute. The chromatography column is then flushed with an Imidazole buffer that binds competitively to the resin and washes out the desired enzyme (Scheme 2). The purified enzyme is collected in fractions.

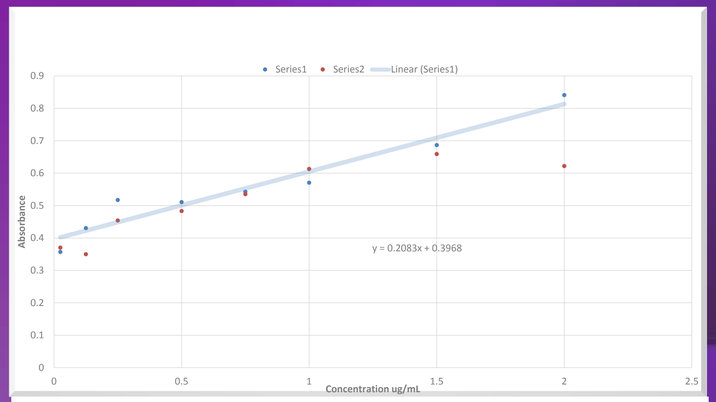


Figure 3: Absorbance of the known enzyme against a standard protein Ovalbumin using Bradford reagent. The standard allows the concentration of the known enzyme to be calculated.

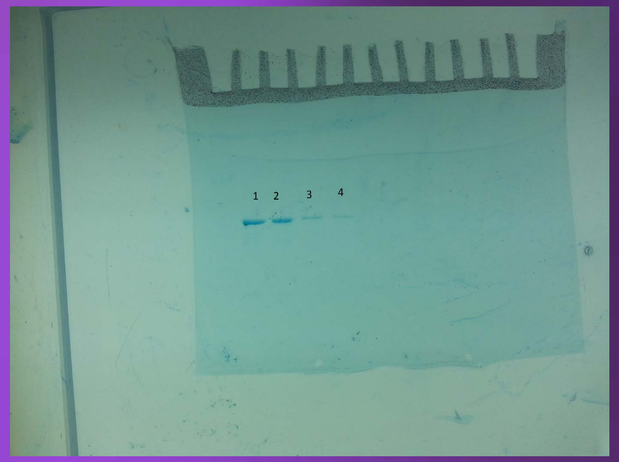


Figure 2: Lanes 1-4, Tyr RS enzyme fractions Following Affinity chromatography.

The enzyme fractions collected from the affinity chromatography then undergoes SDS-Page electrophoresis to determine its purity. This type of electrophoresis allows the enzyme and any impurities to travel down the electrophoresis gel according to molecular weight (Figure 2). The resulting gel is then stained to show the bound enzyme and any impurity present.

The purified enzyme is analyzed using a Coomassie (Bradford) Assay in which the reagent binds with the enzyme. By changing the amount of enzyme allowed to bind with the reagent and measuring its absorbance of light we can find the actual concentration of the enzyme we've purified.

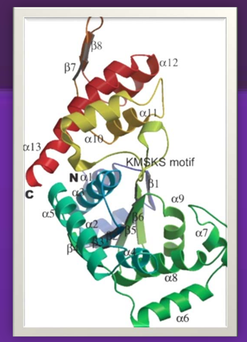


Figure 3: **Tyr RS enzyme found in M. Jannaschii**

The larger goal of our project is to determine exactly how this occurred, but the more immediate goal is to validate a new assay for assessing these enzymes efficiency. To this end, we are using a wild type, or natural, enzyme from the bacteria M. Jannascii to standardize the assay. This enzyme is known and its role in nature is to specifically insert the amino acid tyrosine into every growing protein as it is needed (Figure 1).

References:

- Tang; Tirrell *Biochemistry 2002, p. 10635-10645*
- Wang et. al, Science 2001, p 498
- Tippman and Schultz *Tetrahedron, 2007, p. 6182*