Abstract

Aminoacyl-tRNA synthetases (AARSs) are enzymes that catalyze the covalent attachment of amino acids to their cognate tRNA. This reaction is known as aminoligation of tRNA and is crucial for protein synthesis in all living organisms. These essential enzymes are large proteins, comprised of multiple domains. It has been proposed that the coupled dynamics between various structural elements of these enzymes are responsible for facilitating enzymatic rate enhancement. Unfortunately, previous in vitro studies were limited to dilute solution environments, and were unable to account for the impact of the macromolecular crowding in the cellular environment on these coupled dynamics. We are employing an experimental, non-radioactive enzyme kinetics approach, to probe the impact of macro molecular crowding agents such as sucrose, dextran, and ficoll-70 on the structure, dynamics, and function of Escherichia coli poyl-tRNA synthetase. The preliminary data of our comparative study in the absence and presence of crowding agents will be presented.

METHOD

Non radioactive Kinetic assay (malachite green)

- Aminoligation of tRNA is a two-step reaction:
  \[
  \text{AA + ATP + AARS } \rightarrow \text{AARS-AA-AMP + PP_i} \quad (1) \\
  \text{AARS-AA-AMP + tRNA } \rightarrow \text{AARS + AA-tRNA + AMP} \quad (2)
  \]

- Malachite green interacts with inorganic phosphate along with molybdate resulting in a green color. This color change can be used to measure the rate of amino acid activation and aminoligation of proRS6 in the presence of various macromolecular crowding agents.

- Malachite Green reaction:
  \[
  \text{AA + ATP + AARS } \rightarrow \text{AARS-AA-AMP + PP_i} \\
  \text{PP_i cleavage with PPAse} \\
  \text{PPase + PP_i + H_2O } \rightarrow 2P_i \\
  \text{Malachite Green reaction} \\
  \text{Malachite Green Solution } + 2P_i
  \]

- Absorbance at 620 nm

- General Procedure and Materials
  - With a constant concentration of protein, we first added varying concentrations of sucrose (hydodynamic radius 5.9 Å) and ficoll 70 (hydodynamic radius 40 Å) to simulate the crowded cell environment [7].
  - To determine the kinetic parameters, we added fixed concentrations of proline and ProRS and took out aliquots at various time points.

RESULTS

- Hydrodynamic radius of crowders
  - Absorbance at 620 nm

CONCLUSIONS

- The non-radioactive malachite green assay has been optimized for our system protein.
- The presence and increasing size of hydodynamic radii of macromolecules negatively impacts the kinetics and catalytic efficiency of ProRS.

FUTURE WORK

- Continue studying the impacts of other macromolecules like dextran with different hydodynamic radii.
- Determine K_M and V_MAX for ProRS under macromolecular crowding conditions by varying the substrate concentration in order to compare the catalytic efficiency.

REFERENCES

[1] Schultz et al. (1961) AARS + AA-tRNA + AMP → AARS-AA-AMP + PP_i, (2)

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