Exploring the Interplay of Dynamics and Catalysis in *Escherichia coli* Prolyl-tRNA Synthetase

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ABSTRACT

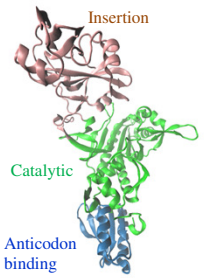
Proteins are intrinsically dynamic. Protein dynamics are known to be critical for many important biochemical processes. However, there is still limited information regarding how dynamics favor enzymes to achieve their enormous rate enhancement. To better understand the molecular mechanism of the interplay between dynamics and catalysis, we are currently involved in modeling a very important biochemical reaction known as aminoacyl-adenylate formation. This reaction is catalyzed by aminoacyl-tRNA synthetases, a family of enzymes that play a crucial role in the protein synthesis of all living organisms. We are using quantum mechanical/molecular mechanical approaches to model and compute energetics of adenylate formation reaction in enzyme and enzyme-free system. Herein, we have presented the preliminary results of our study, which include the free-energy of activation of aminoacyl adenylate formation in aqueous solvent and the QM/MM-treated substrates-bound enzyme system.

BACKGROUND

- **Prolyl-tRNA Synthetases (ProRSs)**
  - Multi-domain, allosterically-regulated enzymes [*1*].
  - Catalyze the covalent attachment of proline to $tRNA^{Pro}$ in a two-step reaction:

    Proline + ATP + ProRS ⇆ Pro-AMP•ProRS + PP_i (1)

    Pro-AMP•ProRS + $tRNA^{Pro}$ → Pro-$tRNA^{Pro}$ + ProRS + AMP (2)
  - ProRS occasionally misactivates alanine.
  - *E. coli* ProRS possesses an editing mechanism to hydrolyze Ala-$tRNA^{Pro}$ [*2-3*].



The insertion domain is an editing domain, it ensures that the correct amino acid is attached to the tRNA.

The catalytic domain is where the aminoacylation reaction takes place.

The anticodon binding domain is responsible for selecting the corresponding tRNA molecule.

- **Protein dynamics and catalysis**
  - Proteins are intrinsically dynamic in nature.
  - ProRS employs coupled dynamics of its domains [*4*] to accomplish its functions.
  - Intrinsic dynamics affects both the height and width of reaction barrier.

OBJECTIVES

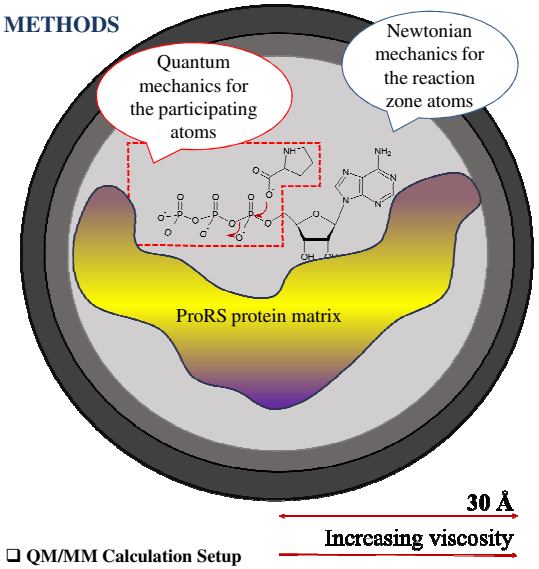
- Computationally determine the activation barrier heights for the following reaction in both aqueous and enzyme-bound states:

  Proline + ATP + ProRS ⇆ Pro-AMP•ProRS + PP_i

- Investigate the effect of protein dynamics on the energy profile
- Identify the role of specific side-chains on catalysis

Acknowledgements-
-Office of Research and Sponsored Programs at University of Wisconsin Eau Claire.
-Blugold Supercomputing Center and LTS at University of Wisconsin-Eau Claire.
-XSEDE Grant (CHE-110018)

METHODS



- **QM/MM Calculation Setup**

  Reaction zone, treated with Newtonian mechanics (up to 24 Å).

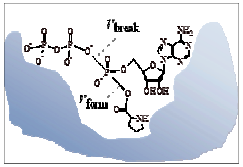
  Buffer zone, treated with Langevin's Dynamics (from 24 to 30 Å) .

  Reservoir zone, atoms here are deleted. Generalized Born's model for solvation using continuum electrostatics are applied (greater than 30 Å).

- **Potentials of Mean Force (PMF)**
  - The free energy change along a reaction coordinate, ξ

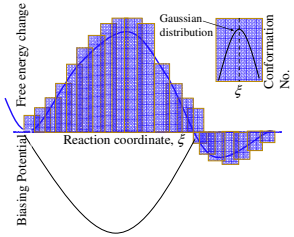
    $\xi = r_{broken} - r_{formed}$ and

    $G(\xi) = -k_B T \ln\langle p(\xi)\rangle$



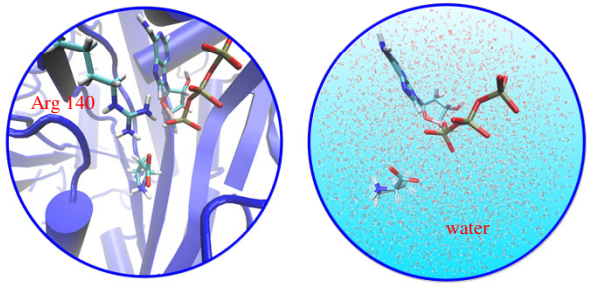
  - k_B and T are Boltzmann's constant and temperature, respectively.
  - $\langle p(\xi)\rangle$ averaged Boltzmann probability distribution with respect to a reaction coordinate [*5-6*].

- **Umbrella Sampling**
  - The range of the reaction coordinate was divided into numerous trials
  - MD simulations were run independently using a unique biasing potential relative to the intermediate state
  - Weighted Histogram Analysis Method (WHAM) was used to obtain free energies



RESULTS

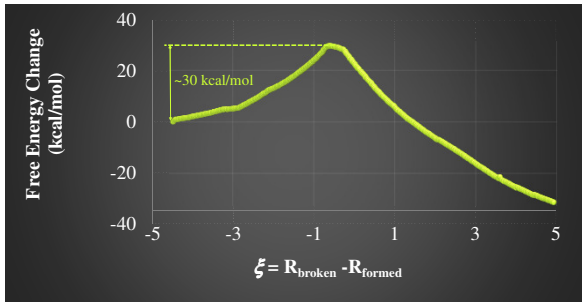
- **'U'-shaped conformation of the ATP**



In ProRS active site

Aqueous state

- **Arg 140 is a key residue to anchor the ATP**
- **A large free energy barrier for the aqueous-state reaction**



CONCLUSIONS

- There is a large activation barrier height of ~30 kcal/mol in the aqueous state.
- ATP acquires an U-shaped conformation prior to the attack by the carboxylate group of the proline
- Arg 140 has been identified with having a key role in anchoring the ATP in the ProRS active site.

FUTURE DIRECTIONS

- To determine the activation barrier height in the ProRS-bound active site.
- Site-directed mutagenesis and enzyme kinetics based on the theoretical model generated in this work.

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