Investigating Structure-Dynamics-Function Differences Among Various Species of Proyl-tRNA Synthetase Using a Hybrid QM/MM Computational Technique

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Abstract

All living organisms contain aminoacyl-tRNA synthetases (AARSs) — a family of enzymes critical for protein synthesis. They are responsible for the correct attachment of amino acids to the specific tRNA molecules called aa-tRNAs, using ATP. The structures of proyl-tRNA synthetase (ProRS) for different species have been found to contain a number of domains. The "prokaryotic-like" species of ProRS consist of a one-binding domain instead of the reaction domain (INS domain present in "eukaryote-like" species). These domains are in constant motion and work as a concerted manner to facilitate the catalytic reaction step. Coupled domain dynamics have been found to be necessary for the synthase. This calls into question the differences in the two non-catalytic domains and how it influences the catalytic step. A comparative study of the structure-dynamics-function relationship among various species of ProRS is being performed using molecular simulation techniques. In parallel, a comparative study of the sequence homology among species of ProRS using the Dali server for the comparison of proteins is being performed. In order to further investigate the evolution of the ProRS family, the simulations and differences of different ProRS species are being tested. The preliminary results for this investigation will be presented.

Function

- Aminoacyl-tRNA synthetase (AARSs)
- Catalyze the covalent attachment of an amino acid (AA) to its cognate tRNA to form aminoacylated-tRNA
- Occurs in two steps: AA + ATP + AARS → AARS•AMP + PP

Structure

Two core domains with sequential functions
- Catalytic domain where covalent attachment of proline to tRNA takes place in the presence of ATP
- Anticodon binding domain responsible for recognition of the correct tRNA

Materials and Methods

- Four layers of water were added with a dynamic simulation in between each step for the protein to adjust to each addition using CHARMM program (version C4061)
- Structure was then re-centered at the active site and truncated to a 60 Å radius sphere

Dynamic

- ProRS dynamics and interactions are considerably different from ion soot
- Role of coupled domain dynamics in catalysis is still underexplored in large, multi-domain enzymes
- Protein structures determined of the INS domain of ProRS of T. Thermophilus and E. coli showed that the INS domain is involved in catalysis and difference in domain dynamics possibly gives rise to the absence of INS domain dynamics
- Work done by the ProRS domains studied demonstrated the involvement of the INS domains in the catalytic domain critical for efficient catalysis

Theory

Quantum Mechanics/Molecular Mechanics (QM/MM) Hybrid Technique

- Why use the hybrid QM/MM technique?
- Quantum theory is required for simulating bond breaking and bond forming but computationally expensive for simulating electrostatics and van der Waals interaction, so it is used to simulate both in multidomain enzymes
- Different regions of the isolated enzyme-substrate complex are treated with different levels of theory describing atoms and atom-atom bonding
- Atomic dynamics are utilized in breaking and formation of bonds and angles
- The rotary and sliding movement in the bipartite phosphatase (MCP-5a) is a result of electronic exchange calculations
- The role of the protein and the water molecules are treated through classical Newtonian Mechanics
- Active pocket for the substrate is not only important for catalysis but also in ligand binding
- Atomistic potentials can be used to study changes in the potential energies of bonds and angles, although not sufficient
- The dynamic interactions are then demonstrated for simulations
- Internal force constants, $C_{ij}$, is the depth of the potential energy, and $f$ is the desorption coefficient

Results and Discussions

- Overall Structural Dynamic Changes
- Comparison of the original structure against the presence of AMP + PP, ATP + tRNA, and INS+amp
- The INS domain is a significant contributor to the active site pocket
- Movement of the INS domain in modified species of E. coli ProRS

Conclusions and Future Directions

- All species exhibit distinct structural dynamics in pre-stressed conditions
- The INS domain movement exhibits reduced flexibility
- The force on INS domain is increased in the presence of AMP + PP
- The INS domain movement is significant in the presence of ATP
- The INS domain movement is a significant contributor to the active site pocket
- Coordination of the INS domain movement is responsible for the catalytic reaction
- The INS domain movement is necessary for the catalytic reaction

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References