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Characterization of *Tte* UvrD Helicase Activity

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Background and Motivations

- UvrD helicases are a class of proteins that unwind DNA and provide critical functions in DNA mismatch repair (MMR) processes.
- While the mechanism surrounding the role of E. coli UvrD in prokaryotic MMR has been well-characterized, the eukaryotic MMR process is less understood due to its complexity.[1,2]
- Recent work has shown that MMR processes in some thermophilic prokaryotes share similarities with eukaryotic processes, making the characterization of thermophile UvrD proteins of key interest.[3]
- Analysis of degree, efficiency, and rate of *Thermoanaerobacter tengcongensis (Tte)* UvrD DNA unwinding was conducted through ATPase, native gel, and FRET assays.

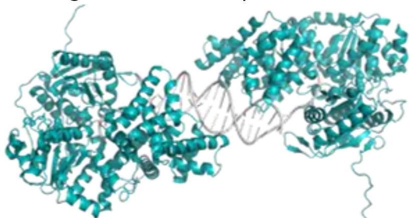


Figure 1. Crystal Structure of *E. coli* UvrD (PDB: 2is6)

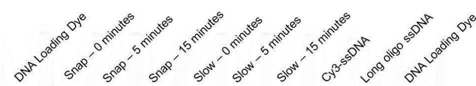
Hypothesis

- Because snap-frozen DNA strands present more mismatch pairs rendering it more unstable, the helicase should unwind the snap-frozen DNA faster and with greater efficiency due to the lower energetic cost.
- Initial velocity as a function of UvrD concentration under saturated ATP is expected to follow pseudo-zeroth order kinetics, while varying ATP concentration is expected to follow standard Michaelis-Menten kinetics.

Figure 2. Comparison of snap frozen (left) to slow annealed (right) DNA substrates with 3' overhang.

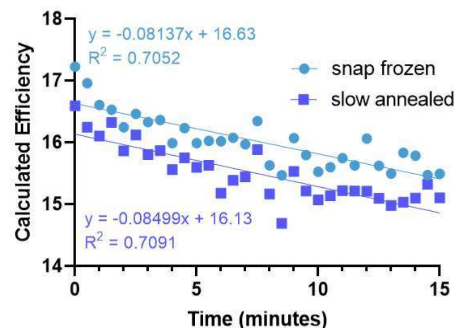
Degree and Kinetics of DNA Unwinding

Figure 3. Native Gel



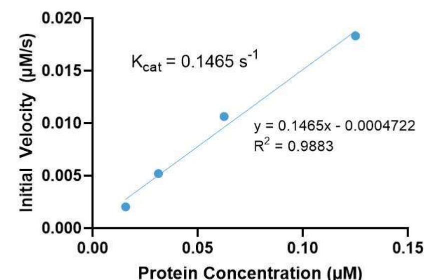
Helicase reactions were performed using snap frozen and slow annealed dsDNA under varying times with ATP. The lighter bands lower on the gel correspond to unwound ssDNA.

Figure 4. FRET Assays



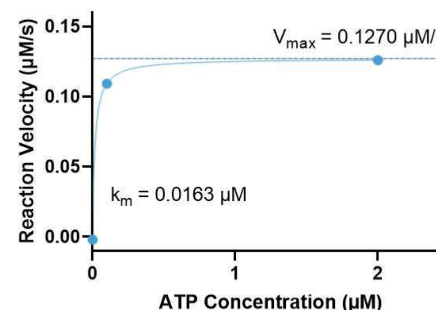
The FRET assay monitored the unwinding of snap frozen and slow annealed dsDNA by *Tte* UvrD in real time. Efficiencies greater than one indicate unwinding data is unreliable.

Figure 5. ATPase Assay Varying Protein Concentration



Tte UvrD follows pseudo-zero order kinetics at saturated ATP conditions.

Figure 6. ATPase Assay Varying ATP Concentration



Michaelis-Menten plot varying ATP concentration at 600 pM UvrD is unreliable due to few usable data points and possible contamination of the samples.

Conclusions

- ATPase assays confirm the ability of *Tte* UvrD to unwind dsDNA.
- *Tte* UvrD follows pseudo-zeroth order kinetics as a function of protein concentration.
- The kinetic characterization of *Tte* UvrD as a function of ATP concentration and the efficiency of separation was inconclusive and nonreliable.

Future Directions

- Use higher protein and ATP concentrations in assays to avoid issues with instrumental limits of detection.
- Characterize UvrD analogs from other thermophilic prokaryotes like *Thermus Aquaticus (Taq)*.
- Probe the function of UvrD in the MMR process instead of typical DNA unwinding.

References

1. Li, G.M. Mechanisms and functions of DNA mismatch repair. Cell. Res. [Online] 2008, 18, 85–98. https://doi.org/10.1038/cr.2007.115
2. Liu, D; et al. DNA mismatch repair and its many roles in eukaryotic cells. Mutation research [Online] 2017, 773, 174-187. https://doi.org/10.1016/j.mrrev.2017.07.001
3. Qiu R; et al. MutL traps MutS at a DNA mismatch. Proc. Natl. Acad. Sci U.S.A. 2015, 35(112), 10914-9. https://doi.org/10.1073/pnas.1505655112