Elucidating the Characteristics of the *Thermus aquaticus* UvrD Helicase

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Introduction

Background

- Thermus aquaticus (*Taq*) UvrD codes for the DNA helicase II protein.
- DNA Helicase II unwinds DNA to perform mismatch repair (MMR), where the incorrect nucleotide is cleaved and replaced by a DNA polymerase III.
- Nucleotide mismatches that are not corrected can lead to mutated proteins and genetic disorders.
- *Taq* utilizes a hybrid system between E.coli and eukaryotic MMR.
  - The similarities include that neither Taq and E.coli UvrD are methyl-directed or MutH homologues
  - The differences include if helicase is necessary for eukaryotic systems although helicases have been shown to interact with eukaryotic MMR proteins.

Hypothesis

The hypothesis is to express and analyze Taq UvrD, a helicase that unwinds and eliminates incorrect DNA, and compare its activity with that of the E. coli UvrD, which is homologous to it.

Findings

- Taq UvrD was not successfully purified using the protocols described, T.te was purchased and used for further experiments.
- Helicase assays did not indicate that T.te was able to unwind DNA.
- T.te FRET assays showed inconclusive evidence.

Conclusion

Our data is not sufficient to achieve our mission statement and thus we cannot compare it to E.coli UvrD MMR pathways.

Significance

The work presented here gives information that can guide future experimental work in this field. The discussed protocols were unsuccessful and require additional research into optimization.

Methods

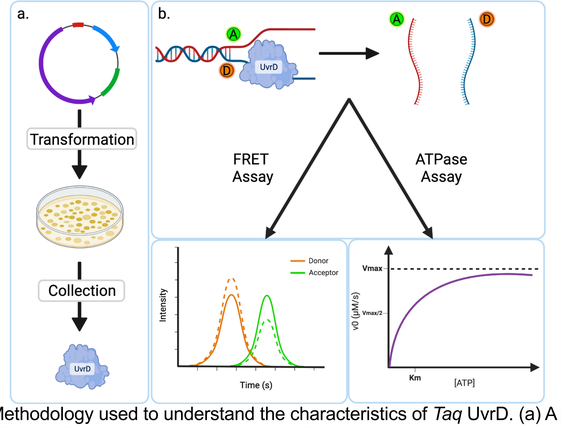

Figure 1. Methodology used to understand the characteristics of *Taq* UvrD. (a) A plasmid containing *Taq* UvrD was first transformed using BL21 cells then the protein was collected and purified. (b) To examine the unwinding efficiency of UvrD, a FRET assay and an ATPase were run twice, once with DNA with a 3' overhang and once with blunt ends.

Results

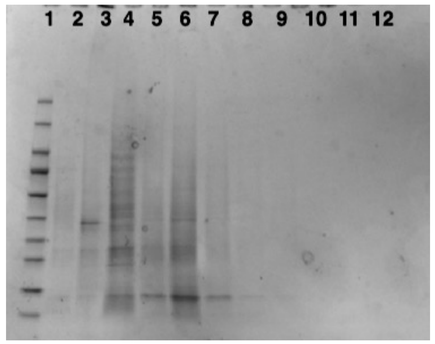

Figure 2. SDS-PAGE gel with ammonium sulfate and column chromatography samples. From left to right: induced (2), insoluble (3), ammonium sulfate supernatant (4), ammonium sulfate pellet (5), talon flow through (6), talon wash (7), talon elute (8), chitin flow through (9), chitin wash (10), chitin elute (11), and purified chitin elute (12). The lack of banding in the elutes indicates that the protein was not retained by the column. We use purchased T*hermoanaerobacter tengcongensis (T. te)* UvrD for further experiments.

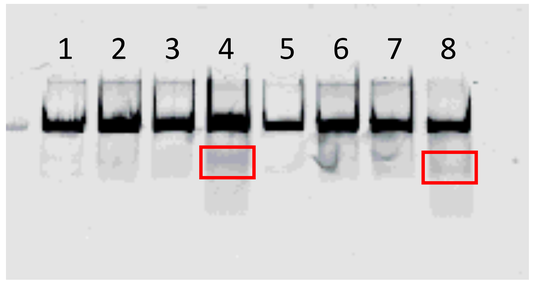

Figure 3. Helicase assay gel. From left to right: lanes 1-4 are the control samples with no T.te UvrD added (room temperature (RT), 40°C, 70°C, 90°C); lanes 5-8 are the experimental samples at RT, 40°C, 70°C, and 90°C. There is a faint secondary band present in lane 8, indicating that some of the DNA was successfully unwound. However, a secondary band is also present in lane 4 for the 90°C control, which indicates this may not be due to UvrD.

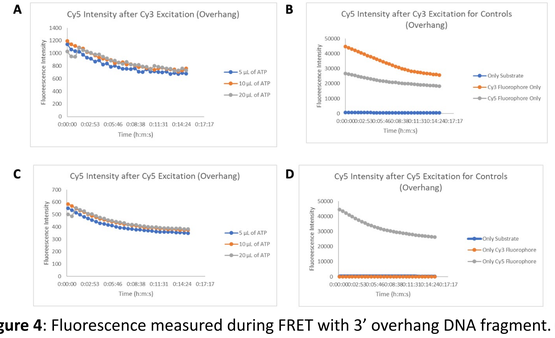

Figure 4: Fluorescence measured during FRET with 3' overhang DNA fragment. (A) Fluorescence of Cy5 fluorophore at 5, 10, or 20μl of ATP. As the reaction continues, there is a slight decrease in acceptor intensity over time. (B) Fluorescence of Cy5 fluorophore at 5, 10, or 20μl of ATP. The donor intensity decreases steadily over time before plateauing. (C) Fluorescence of Cy5 fluorophore for controls. (D) Fluorescence of Cy5 fluorophore for controls.

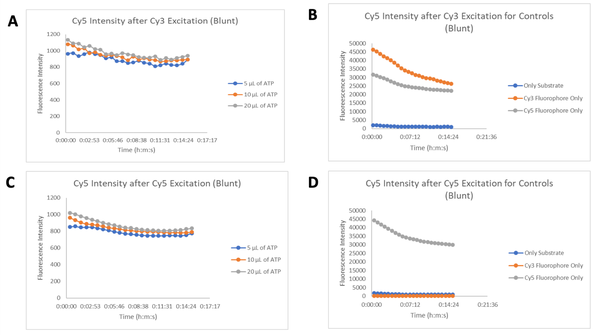

Figure 4: Fluorescence measured during FRET with blunt end DNA fragment. (A) Fluorescence of Cy5 fluorophore at 5, 10, or 20μl of ATP. (B) Fluorescence of Cy5 fluorophore at 5, 10, or 20μl of ATP. In both A and B, the intensity decreases steadily over time before increasing slightly at the end. (C) Fluorescence of Cy5 acceptor fluorophore for controls. (D) Fluorescence of Cy5 fluorophore for controls.

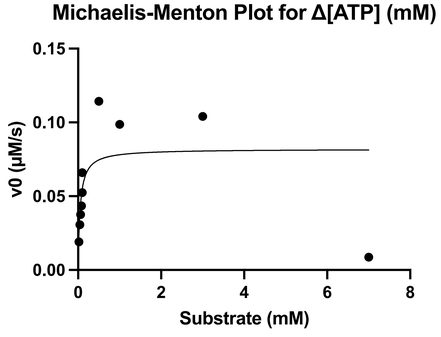

Figure 5: Michaelis-Menton plot for varying ATP concentrations. The Michaelis-Menton curve for the control samples with substrate, ATP, concentrations at 0.02, 0.04, 0.06, 0.08, 0.1, 0.5, 1.0, 3.0, and 7.0 mM.

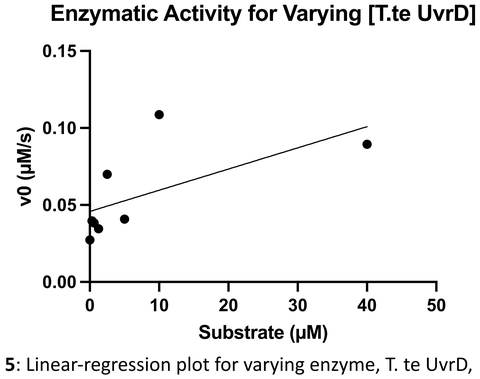

Figure 5: Linear-regression plot for varying enzyme, T. te UvrD, concentrations. The Michaelis-Menton curve for the control samples with substrate, ATP, concentrations at 0.02, 0.04, 0.06, 0.08, 0.1, 0.5, 1.0, 3.0, and 7.0 mM.

Conclusions

- Notable error in obtaining protein using cellular transformation and restriction digest resulting in studying T.te UvrD instead.
- ATPase assay absorbance for higher concentrations of ATP or protein was lower than that of lower concentrations.
- Helicase assays did not indicate that T.te was able to properly unwind the DNA
- FRET assay indicated that the fluorescence intensity decreased initially and then began to plateau over time.
- Future work should further investigate the function of UvrD in cellular mismatch repair by investigating the effect of temperature on UvrD activity.
- Overall, the data obtained provides valuable information for further experiments to better understand the characteristics of UvrD.

Future Directions

- Due to limited time and limited protein amounts, limited samples were made.
- The characteristics of T.te UvrD may differ slightly from those of Taq UvrD.
- Future work should utilize the desired *Taq* UvrD rather than substitution T.te to better understand the characteristics of the helicase
- In the future, more sample points should be taken to develop more accurate trend lines, particularly with the Michaelis-Menten curve.

References

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