The characterization of helicase activity and enzyme specificity of *Tte*-UvrD

THE UNIVERSITY *of* NORTH CAROLINA *at* CHAPEL HILL

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Introduction

Background

- UvrD (helicase II), an essential enzyme in DNA mismatch repair (MMR), is required in many prokaryotic species.[1]
- Although UvrD is required in the methyl-directed MMR pathway in *Escherichia coli* (*E. coli*), it is unknown whether helicases are required in eukaryotic MMR.[1]
- MutH-deficient prokaryotic organisms are theorized to provide a hybrid model between eukaryotic and *E. coli* MMR.[2]
- UvrD from MutH-deficient thermophilic species such as *Thermus aquaticus (Taq)* and *Thermoanaerobacter tengcongensis (Tte)* is readily accessible to characterize *in vitro.*[3]

Figure 1. MMR pathway comparisons

	E. coli	*Taq/Tte*	Eukaryotes
Methyl-directed	✓	✗	✗
MutS/MutSα	✓	✓	✓
MutL/MutLα	✓	✓	✓
MutH	✓	✗	✗
UvrD/helicase	✓	✓	?

Figure 1. Comparisons of MMR pathways in E. coli, MutH-deficient prokaryotes (most prokaryotes; *Tte/Taq*), and eukaryotes.[2]

Hypothesis

The unwinding velocity and ATPase activity of *Tte*-UvrD is comparable to that of *E. coli*-UvrD at their corresponding optimal temperatures (*Tte*: 65˚C; *E. coli*: 37˚C).

Findings

- The native gel helicase assay unsuccessfully demonstrates the helicase activity of *Tte*-UvrD (0% unwound DNA) **(Figure 2)**.
- *Tte*-UvrD successfully unwinds substrates with 43 base overhangs (3' end) **(Figures 3 and 4).** ATP concentrations ranging from 25-100 uM result in comparable levels of FRET efficiency loss and fluorophore distance gain over 10 minutes.
- *Tte*-UvrD is incapable of unwinding blunt end DNA, regardless of ATP concentration **(Figure 5)**.
- *Tte*-UvrD was found to have a K_m of 0.2761 uM and V_{max} of 0.1551 uM/s **(Figure 6).** K_{cat} was determined to be 2.7304 s^{-1} **(Figure 7)**.

Significance

Comparability, or lack thereof, of ATPase activity between *E. coli*-UvrD and *Tte*-UvrD aids in the understanding of the role of helicases in eukaryotic MMR through the proposed hybrid model. Such information is critical to further research MMR deficiencies in humans.

Methods

Transformation, Expression, and Purification (*Taq*-UvrD)

1. Heat Shock
2. Autoinduction

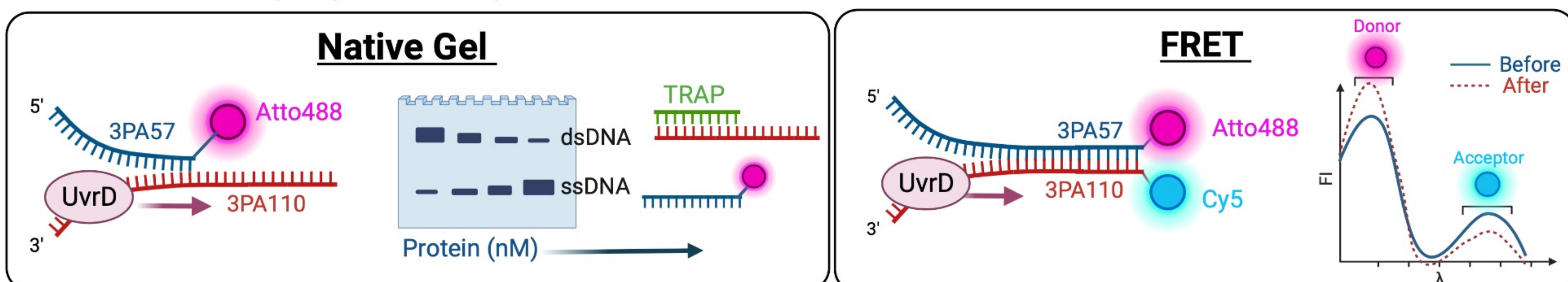
→

1. Cell lysis
2. Ammonium sulfate precipitation

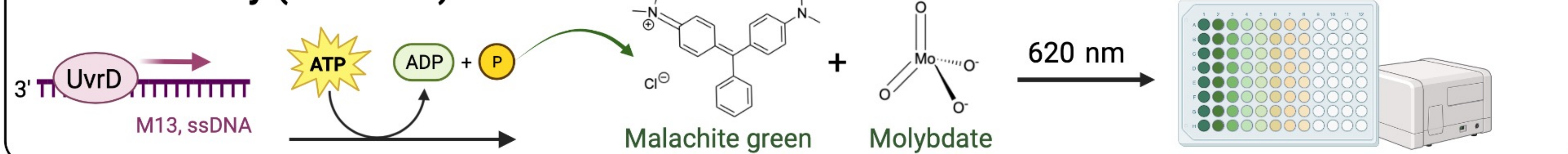
→

1. His-tag affinity chromatography
2. Chitin column

Helicase Assays (*Tte*-UvrD)



ATPase Assay (*Tte*-UvrD)



Results

Figure 2. Native gel helicase assay

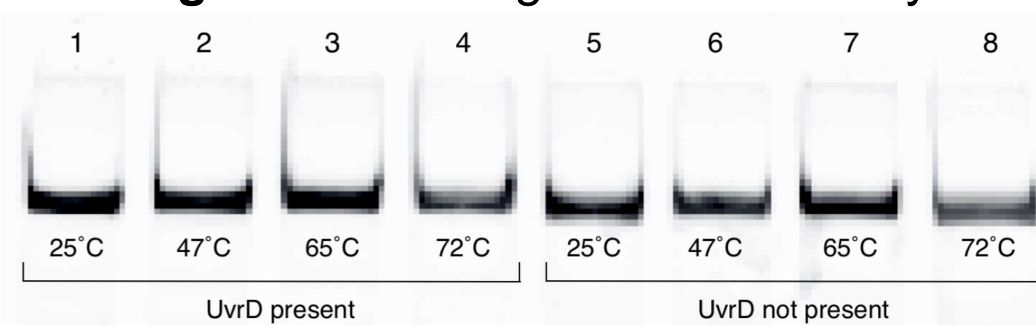


Figure 2. TBE-PAGE of helicase reaction samples as described in *Materials and Methods* and imaged using BioRad Gel Doc EZ System software. Lanes 1-4 depict samples containing *Tte*-UvrD. Lanes 5-8 depict controls without *Tte*-UvrD. Lanes 1 and 5: 25˚C; Lanes 2 and 6: 47˚C; Lanes 3 and 7: 65˚C; Lanes 4 and 8: 72˚C. All samples depict 0% unwound ssDNA (100% dsDNA).

Figure 3. FRET efficiency (3' overhang)

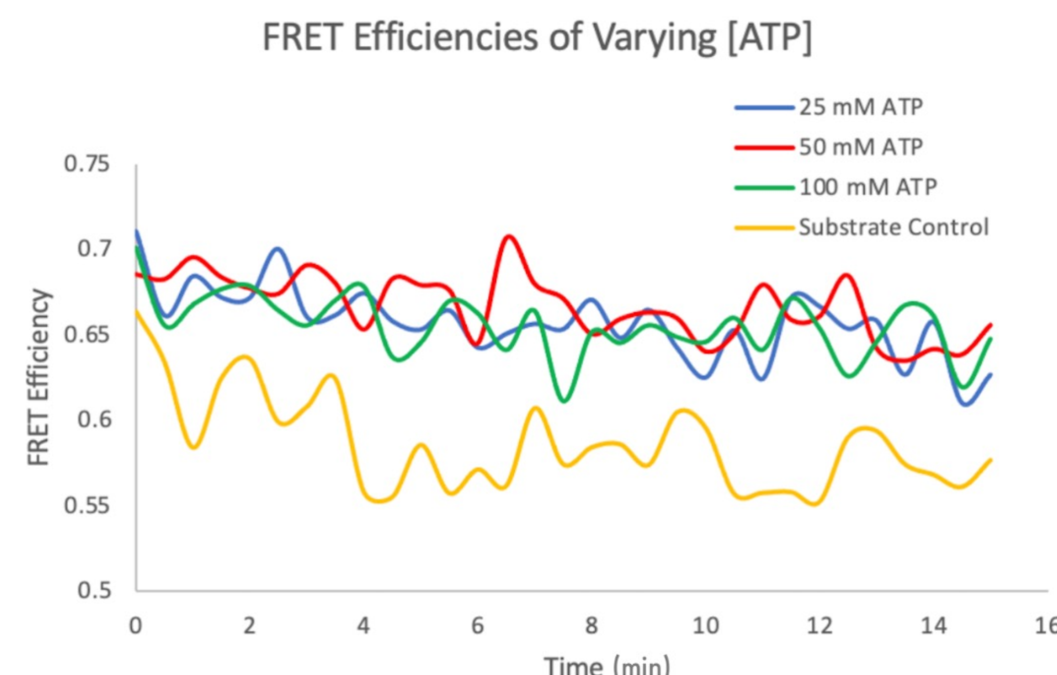


Figure 3. FRET efficiency of containing 3' overhang DNA and varying amounts of ATP gathered via FRET Assay. Substrate control contains 3' overhang DNA only. Fluorescence data was collected via a Biotek Synergy HTX plate reader.

Figure 4. Fluorophore distance (3' overhang)

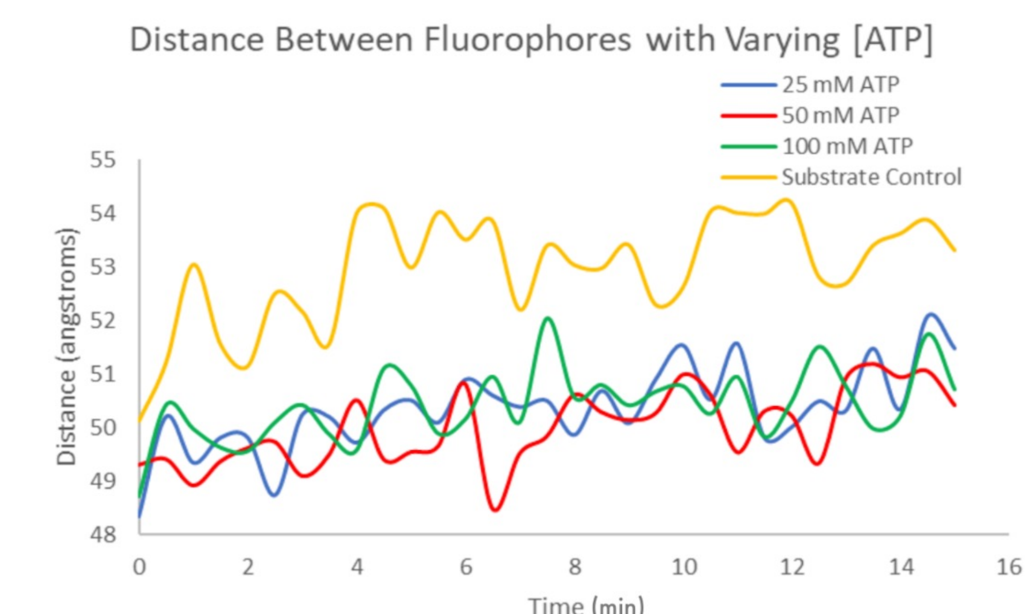


Figure 4. Distance between fluorophores over the course of the FRET helicase assay. Fluorescence data was collected via a Biotek Synergy HTX plate reader.

Figure 5. Normalized FI (blunt end)

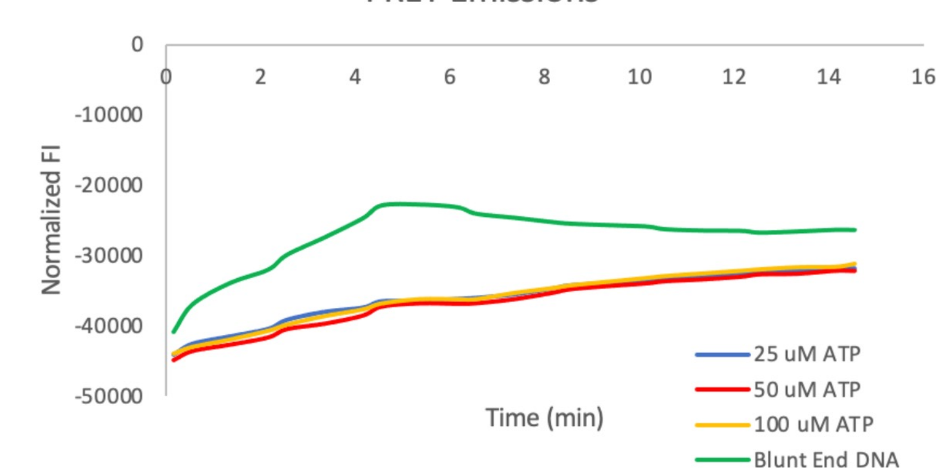


Figure 5. Normalized fluorescence intensities of samples containing blunt end DNA gathered via FRET Assay. Fluorescence data was collected via a Biotek Synergy HTX plate reader.

Figure 6. Michaelis-Menten plot

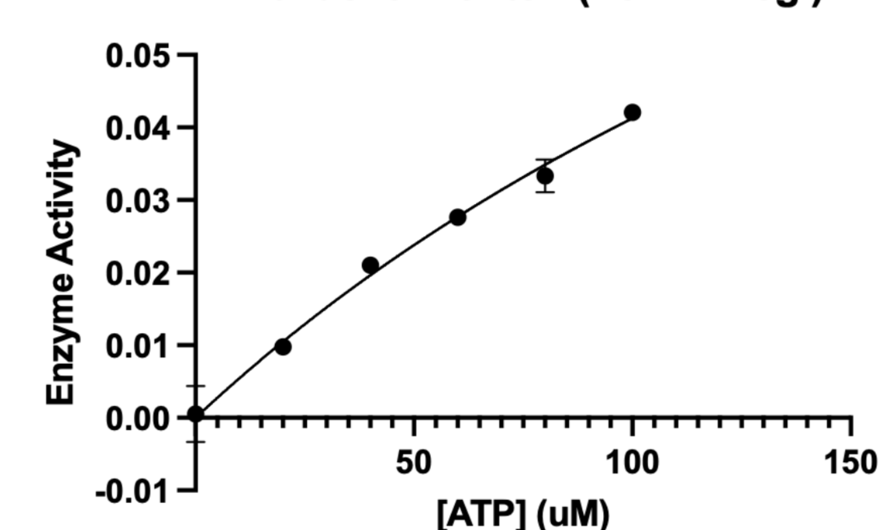


Figure 6. Michaelis-Menten plot of ATPase assay samples (generated via Prism GraphPad). Enzyme concentration remained constant at 0.006024 uM and ATP concentration varied (0, 20, 40, 60, 80, 100 uM). The correlation coefficient (R^2) was 0.9860 with 10 degrees of freedom. Km= 276.1 uM, Vmax= 0.1551 uM/s, Kcat= 25.75 s^{-1}. Enzyme velocity was calculated using the linear regression of phosphate standards to approximate phosphate concentrations after ATP hydrolysis (600 seconds)

Figure 7. Determination of K_{cat}

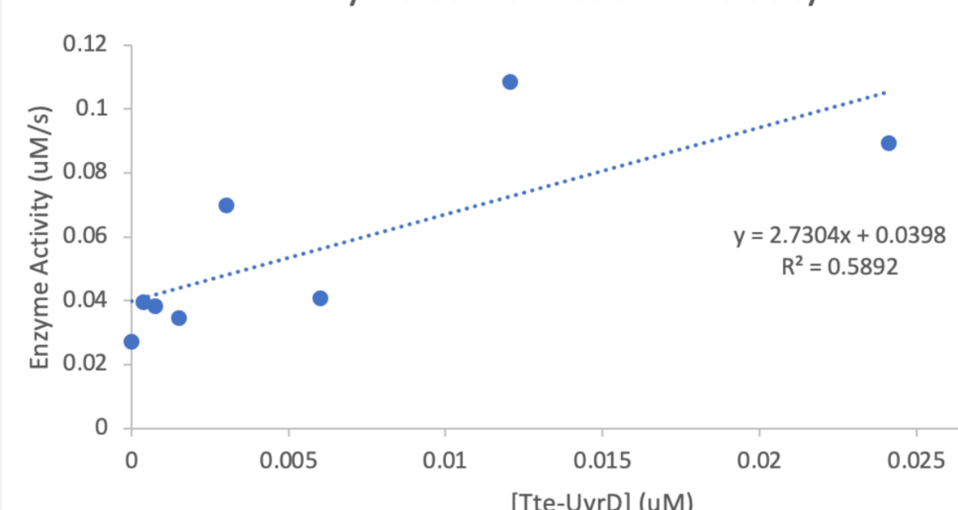


Figure 7. The enzyme velocity of samples of varying enzyme concentrations (0, 0.3765, 0.7530, 1.506, 3.012, 6.024, 12.05, 24.10 nM *Tte*-UvrD). Enzyme velocity was calculated using the linear regression of phosphate standards to approximate phosphate concentrations after ATP hydrolysis (10 minutes) Kcat= 2.7304 s^{-1}.

Figure 8. Kinetic comparisons of *Tte*-UvrD and *E. coli*-UvrD

Kinetic Parameter	*Tte*- UvrD	*E. Coli*- UvrD[4]
K_m (uM)	0.2761	0.110*
V_{max} (uM/s)	0.1551	0.000021*
K_{cat} (s^{-1})	2.73	147*

Figure 8. Comparison of enzyme kinetics of UvrD from *Tte* and *E. coli*. Data from Tte-UvrD was gathered from the ATPase assays above (linear M13 substrate, incubated at 65˚C). *E. coli*-UvrD data is sourced from a published study of similar conditions (linar M13 substrate, incubated at 37˚C).

*Data was not gathered by the authors of this work; sourced from a previously published article.[4]

Conclusions

- *Tte*-UvrD is capable of unwinding 3' overhang DNA (43 bases) but is incapable of unwinding blunt end DNA.
- *Tte*-UvrD consumes fewer ATP molecules on a per enzyme basis than *E. coli*-UvrD at their respective optimal temperatures.
- *Tte*-UvrD and *E. coli*-UvrD have comparable affinities for ATP at their optimal temperatures, though *Tte*-UvrD has a lower specificity and catalytic efficiency.

Future Directions

Chain length

We suggest that substrate length be varied in future experimentation to further investigate the translocation ability and processivity of thermostable UvrD prior to dissociation.

Substrate configuration

We suggest that circular ssDNA, G-guadruplexes, and 5' overhang substrates be investigated to further understand possible limitations to thermophilic UvrD.

ATPase activity inhibitors

Investigating the inhibition of ATPase activity by SSB (or other proteins) is essential to create a comprehensive understanding of the role of thermophilic UvrD in its respective MMR pathway.

Temperature dependence

Further studies should be performed to determine whether enzyme kinetics improve and/or helicase activity increases at higher or lower temperatures.

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References

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