Characterizing UvrD Helicase in *Thermus aquaticus*

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Introduction

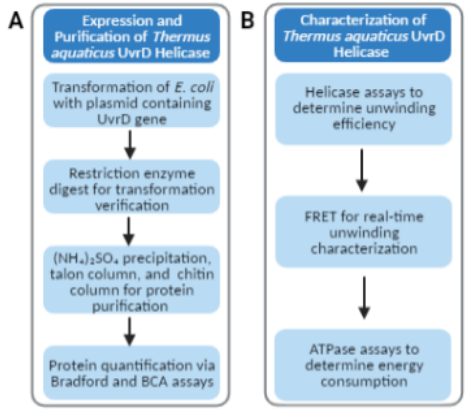
Helicases are a class of ATP-dependent enzymes whose function is to unwind DNA, which allows DNA polymerase to access the strands for replication. Helicases are also involved in DNA repair from errors made by DNA polymerase[1].

DNA Mismatch Repair (MMR) is a critical pathway that corrects mismatched base pairs that arise during DNA replication[2]. Through studies performed using *E. coli*, the prokaryotic MMR mechanism is known, and it has been shown that UvrD helicase, a superfamily I DNA helicase, is essential in nucleotide excision repair and MMR[3].

The MMR helicase activity of eukaryotes is unknown and it is hypothesized to be similar to MMR UvrD activity in prokaryotes. Eukaryotic MMR has the potential to be examined through the use of *Thermus aquaticus* UvrD, which was selected because it is a thermostable hybrid between prokaryotes and eukaryotes[4].

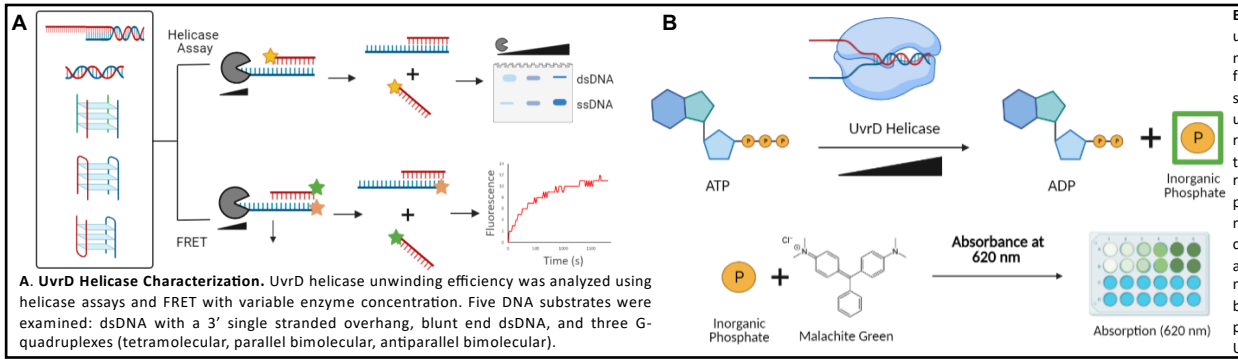
The goal is to study the thermostable *T. aquaticus* UvrD, which is homologous to *E. coli* UvrD, in order to better understand helicase mechanistically in eukaryotic MMR. Although the role of helicase in eukaryotic MMR is unknown, it is important to study because defects in MMR in eukaryotes have been linked to hereditary non-polyposis colorectal cancer[5].

Project Overview



Project Overview Scheme. A. Summary of steps taken for the expression and purification of *Thermus aquaticus* UvrD helicase. **B.** Summary of techniques used to characterize *Thermus aquaticus* UvrD helicase.

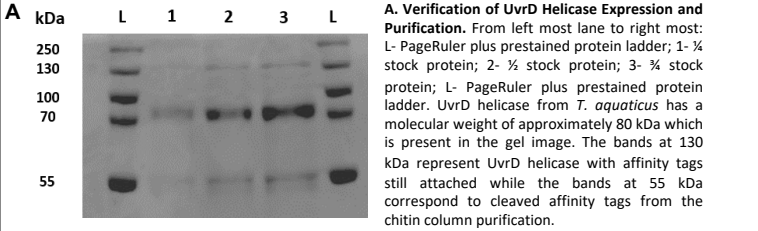
Methods



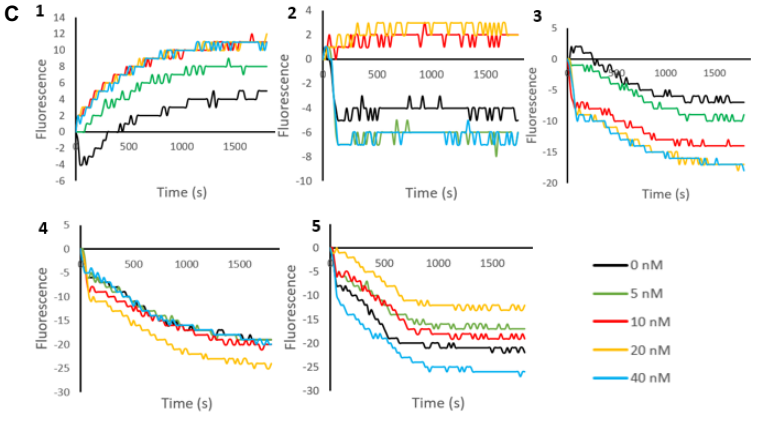
A. UvrD Helicase Characterization. UvrD helicase unwinding efficiency was analyzed using helicase assays and FRET with variable enzyme concentration. Five DNA substrates were examined: dsDNA with a 3' single stranded overhang, blunt end dsDNA, and three G-quadruplexes (tetramolecular, parallel bimolecular, antiparallel bimolecular).

B. ATPase Assay. The assay used in this experiment measures the amount of free inorganic phosphate in solution. As UvrD helicase unwinds dsDNA, energy is required and obtained from the cleavage of ATP. The resulting free phosphate product can interact with malachite green, creating a colored solution that has an absorbance at 620 nm. The measured absorbances can be used to obtain kinetic parameters to characterize UvrD helicase.

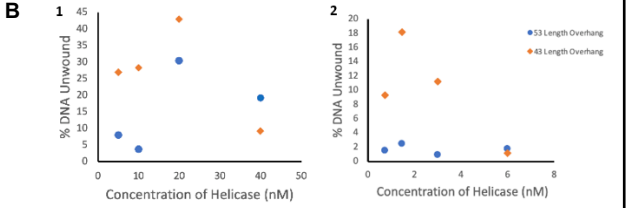
Results



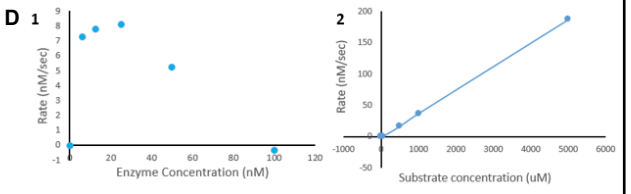
A. Verification of UvrD Helicase Expression and Purification. From left most lane to right most: L- PageRuler plus prestained protein ladder; 1- ¼ stock protein; 2- ½ stock protein; 3- ¾ stock protein; L- PageRuler plus prestained protein ladder. UvrD helicase from *T. aquaticus* has a molecular weight of approximately 80 kDa which is present in the gel image. The bands at 130 kDa represent UvrD helicase with affinity tags still attached while the bands at 55 kDa correspond to cleaved affinity tags from the chitin column purification.



C. Impact of UvrD helicase concentration on fluorescence during FRET analysis of DNA substrates. Fluorescence of donor fluorophore was measured over 30 minutes. Five DNA substrates were examined: **1)** 43 base pair overhang dsDNA; **2)** blunt end dsDNA; **3)** tetra molecular G quadruplex DNA; **4)** parallel bi-molecular G-quadruplex DNA; **5)** antiparallel bi-molecular G-quadruplex DNA. Data for each concentration of UvrD helicase was normalized and plotted against time.



B. *T. aquaticus* and *T. tengcongensis* UvrD helicase unwinding efficiency as a function of concentration. 1) dsDNA containing overhang lengths of 43 (circle) and 53 (diamond) nucleotides were unwound with varied concentrations of *T. aquaticus* UvrD helicase. **2)** dsDNA containing overhang lengths of 43 (circle) and 53 (diamond) nucleotides were unwound with varied concentrations of *T. tengcongensis* UvrD helicase. Percent DNA unwound determined based on gel band intensities.



D. Characterization via ATPase assay. Phosphate concentration calculated through measured absorbance and standard curve. Data normalized by subtracting out phosphate concentration calculated, from the sample containing 0 nM of enzyme. **1)** *T. aquaticus* UvrD helicase ATP energy consumption examined with variable enzyme concentration. Rate calculated by dividing reaction time (20 minutes) from measured free phosphate concentration. **2)** *T. aquaticus* UvrD helicase energy consumption with variable substrate (ATP) concentration and constant enzyme concentration (20 nM). Rate was calculated by dividing reaction time (10 minutes) from measured free phosphate concentration.

Conclusions

Blunt end dsDNA, tetramolecular G quadruplex DNA, parallel bimolecular G quadruplex DNA, and anti bimolecular G quadruplex DNA substrates were unable to be unwound by *T. tengcongensis* and *T. aquaticus* UvrD helicase.

The 43 and 53 base pair overhang dsDNA substrates demonstrated unwinding by *T. tengcongensis* and *T. aquaticus* UvrD helicase, however, percent unwinding and enzyme concentration displayed no consistent correlation.

FRET analysis suggests that there could be a potential conformation change occurring within the G-quadruplex DNA substrates that allows for the donor and acceptor fluorophores to get closer together.

ATPase assay data displayed that the rate of *T. aquaticus* helicase initially increased as enzyme concentration increased, before leveling off and decreasing, suggesting an issue with substrate saturation.

Varying [ATP] resulted in initial fluctuations of rates before concluding with a more linear relationship. This was conflicting to the rate leveling off in the expected sigmoidal pattern, which ultimately prevented the kinetic parameters of *T. aquaticus* helicase from being reported.

Future Directions

Future studies will serve to improve data acquisition for more accurate kinetic analysis.

The conclusions made from the characterization of *T. aquaticus* UvrD helicase in unwinding various DNA substrates serves as a foundation for future research into MMR pathways in eukaryotes.

With a deeper understanding of the function of eukaryotic UvrD helicase via the *T. aquaticus* homolog, future research can focus on developing novel treatments that target MMR defects.

References

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Acknowledgements

Equal contributions were made between group members. We would like to thank all TAs who contributed to the data collection process, as well as both Dr. Freemans for their organization efforts.