Purification and Characterization of Thermostable DNA Helicase II from *Thermus Aquaticus*

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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Introduction

Research Summary

Background

- The unidirectional and thermostable UvrD helicase of *thermus aquaticus* is a DNA unwinding protein that is relevant in the mismatch repair pathway, which is crucial to DNA stability and efficacy, as errors in DNA replications are often the cause of many cancers and disorders.[1]
- Previous studies investigating UvrD in E.coli have revealed interesting insight into the activity of this helicase. Therefore, understanding the similarities and differences in the mechanisms of UvrD in these two bacteria will likely provide insight that can be applied to the eukaryotic MMR process, but little research has been performed on T. aq UvrD.
- This paper investigates the activity of T. aq UvrD and compares it to the homologous E.coli UvrD activity.

Hypothesis

Because Thermus Aquaticus is an extremophile, we predict that unwinding activity will be higher than the E. coli UvrD homolog since it is more stable. Also, because of its similarity to E. coli UvrD, T. aq UvrD will be able to only unwind substrates with a 3' overhang

Findings

- Taq UvrD was successfully expressed and purified from using the techniques represented in this project
- Taq UvrD was able to efficiently unwind DNA with a 3' overhang.
- Kinetic parameters for Taq or E. coli UvrD were not able to be determined, however the T. aq UvrD did show ATPase activity.

Conclusion

Taq UvrD unwound DNA with 3' overhang efficiently, but was not able to unwind any other of the DNA substrates.

Significance

This work helps to better understand the mechanism of action of Taq UvrD. This helicase is an attractive model for building a greater understanding of the MMR pathways in eukaryotic cells due to ket similarities between the two systems.

Methods

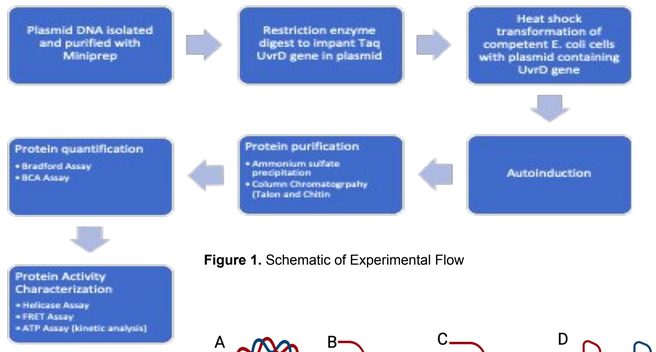

Figure 1. Schematic of Experimental Flow

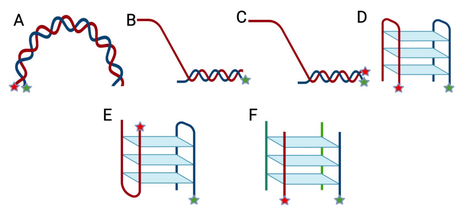

Figure 2. DNA substrates used in Helicase Activity Assays. Stars Represent Radiolabeled ends of DNA substrates.

Helicase/FRET Assay

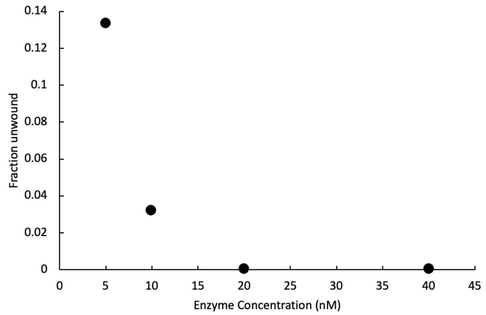

Figure 3. Taq UvrD activity assay on dsDNA substrate with blunt ends. The fraction of dsDNA substrate with blunt ends unwound by varying Taq UvrD concentrations was observed to decrease as enzyme concentration increased. The maximum fraction unwound by Taq UvrD was 0.13.

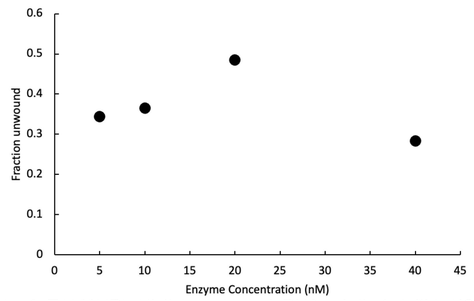

Figure 4. Taq UvrD activity assay on dsDNA substrate with a 43 nt 3' overhang. The fraction of dsDNA substrate with a 43 nucleotide overhang on the 3' end unwound by Taq UvrD is shown. The unwinding activity increased with increasing enzyme concentration until maximum activity was reached at an optimal enzyme concentration, in this case 0.49 unwound at 20 nM enzyme. At the enzyme concentration higher than the optimal, the fraction unwound decreased.

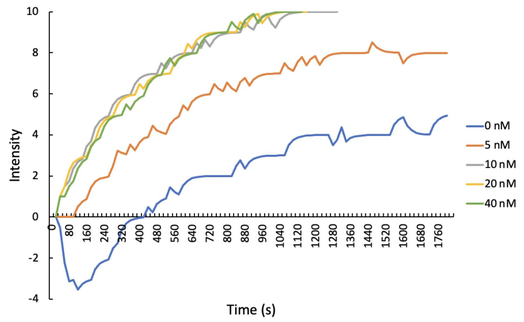

Figure 5. Taq UvrD FRET analysis on DNA substrate with a 43 nt 3' overhang.Increase in donor intensity represents separation of donor and acceptor fluorophores. An increase in intensity was observed for all concentrations with 20 nM and 40 nM having greatest increase. Also, there was a positive correlation between UvrD concentration and helicase activity, with 0 nM showing minimal activity and increase

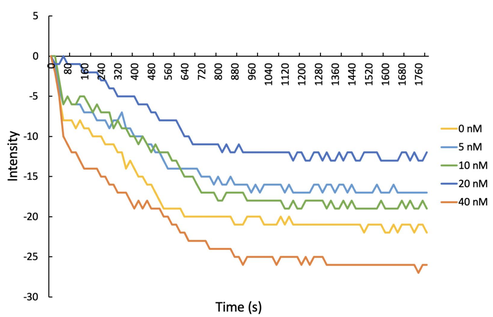

Figure 5. Taq UvrD FRET analysis on Anti-Parallel Bimolecular G Quadruplex DNA substrate. No increase in donor intensity is representative of little to no unwinding.

ATPase Assay

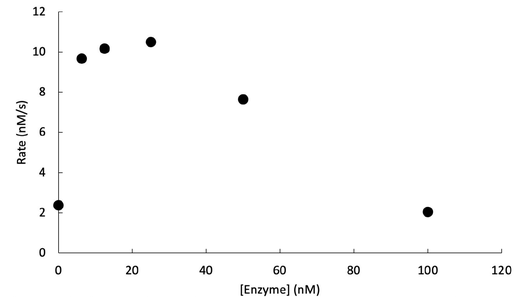

Figure 6. Varying T.aq UvrD Concentration vs Rate of ATP Hydrolysis. Analysis of ATPase assay performed observing the effect of various T.aq UvrD concentrations on the rate of ATP hydrolysis by the enzyme. Michaelis-Menton kinetic values were not able to be extrapolated from this figure since it did not follow a linear fit.

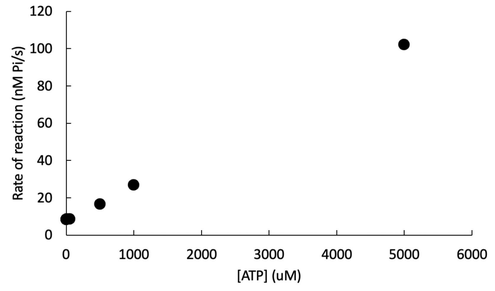

Figure 7. Varying ATP Concentration vs Rate of ATP Hydrolysis by *T. aq* UvrD. Analysis of ATPase assay performed observing the effects of different ATP concentrations on the rate of ATP Hydrolysis by the *T. aq* UvrD enzyme.

Conclusions

- ATPase assays were conducted, but the results of these assays were inconclusive
- Taq UvrD was not able to unwind DNA substrates without an overhang. This is not in agreeance with some previous studies investigating other thermostable UvrD.[2]
  - Likely due to higher temperatures of previous literature causing DNA to vibrate.
- Taq UvrD was not able to unwind superstable G-quadruplex substrates.
- Taq UvrD had higher efficiency with 43 nt overhang compared to a 53 nt overhang.
- Taq did not display logarithmic growth of enzyme activity in relation to enzyme concentration.

Future Directions

- Determine the optimal overhang length
- Determine effects of temperature on helicase activity, including determining an optimal temperature for the enzyme
  - effect of DNA vibrations due to higher temperatures
- Further analysis of the kinetics of the ATP hydrolysis by the enzyme
- Further analysis of how effectively Taq UvrD unwinds certain DNA substrates
  - Immediately perform activity assays after purification to negate freezing the proteins and possibly affecting their activity
- Observing interaction with other proteins in MMR pathway and their effects on Taq UvrD function

References

1. Chung, D. C., & Rustgi, A. K. (1995). DNA mismatchrepair and cancer.Gastroenterology, 109(5), 1685-1699.
2. An, L., Tang, W., Ranalli, T. A., Kim, H. J., Wytiaz, J., & Kong, H. (2005). Characterization of a thermostable UvrD helicase and its participation in helicase-dependent amplification. The Journal of biological chemistry, 280(32), 28952–28958. https://doi.org/10.1074/jbc.M503096200

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