Taq UvrD Helicase DNA Substrate Specificity

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Background

UvrD helicase interacts with damaged and nicked DNA, unwinding segments of DNA for subsequent repair.[1] The DNA which a helicase such as UvrD may act on can include blunt ended double stranded DNA, DNA with a single stranded overhang, and DNA in non-canonical conformations such as G-quadruplexes. Helicase from specific families may preferentially unwind specific DNA substrates[2]. Thermus aquaticus (Taq) is a known thermophile which expresses UvrD helicase; however, Taq UvrD helicase is not well characterized.

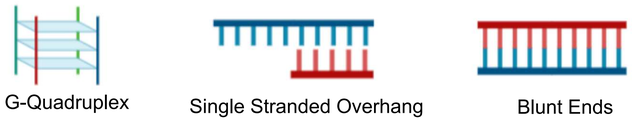

Figure 1. Representations of the DNA substrate types.

In this investigation we will examine the preferential activity of Taq UvrD helicase on a variety of DNA substrates. We anticipate based on previously published findings from E. coli UvrD that Taq UvrD helicase will preferentially unwind DNA with a single stranded overhang in addition to DNA with blunt ends over DNA in any G-quadruplex conformation.[2]

Taq UvrD was successfully expressed and purified. The DNA substrate preference of Taq UvrD was found to be single stranded overhang DNA substrates when examined using helicase and ATPase assays.

Conclusions

- Taq UvrD helicase was able to unwind the 43 and 53 base pair overhangs. Unwinding activity was most efficient at an enzyme concentration of 10 nM (Figure 6). Concentrations above that showed similar unwinding activity.
- The blunt ends and the tetramolecular, antiparallel, and parallel G-quadruplexes were unsuccessful in being unwound by Taq UvrD at any concentration as seen in Figures 4 and 5.
- Kinetic Parameters were able to be calculated for Taq UvrD and k_{cat} was found to be 0.76 s^{-1} (Table 1).

Future Directions

G-quadruplexes helicase preference:[3]

- In vivo consequences
- Location evolutionarily conserved
- Encoding of vital information
- Mis-regulated structure can be detrimental

These findings indicate that only highly specialized helicase may be able to interact with G4 DNA substrate.[4] What characteristics make a helicase preferentially process G4 substrate?

Methods

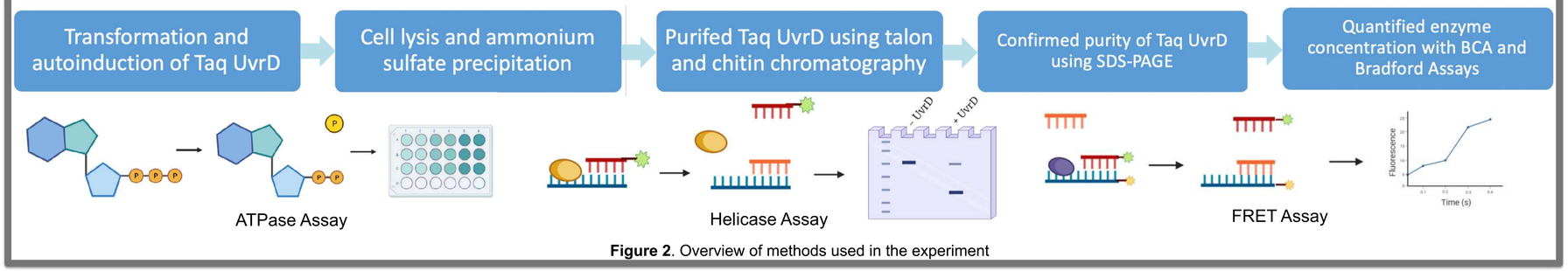

Figure 2. Overview of methods used in the experiment

Results

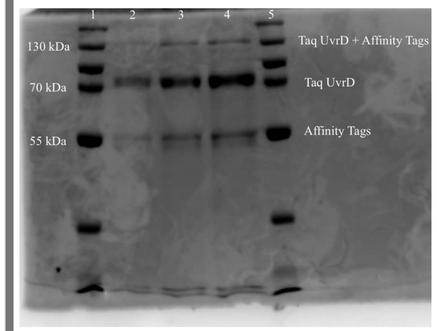

Figure 3: SDS-PAGE Gel of Protein Purification. Lanes 1 and 5 are molecular weight ladders. Lanes 2, 3, and 4 are the diluted proteins at ¼ , ½ and ¾ of our eluted protein.

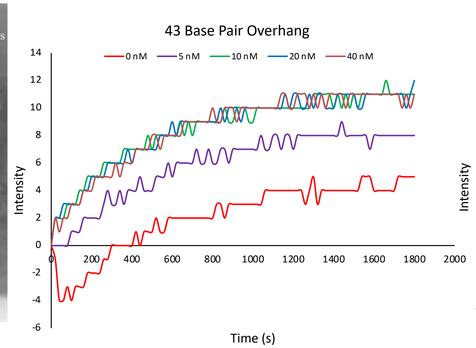

Figure 6 : Graph of Intensity vs Time of the FRET Data for 43 Base Pair Overhang DNA Substrate with varying *Taq* UvrD concentrations of 0, 5, 10, 20, 40 nM.

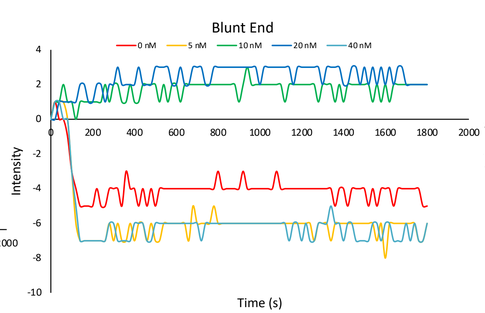

Figure 7 : Graph of Intensity vs Time of the FRET Data for Blunt DNA Substrate with varying *Taq* UvrD concentrations of 0, 5, 10, 20, 40 nM.

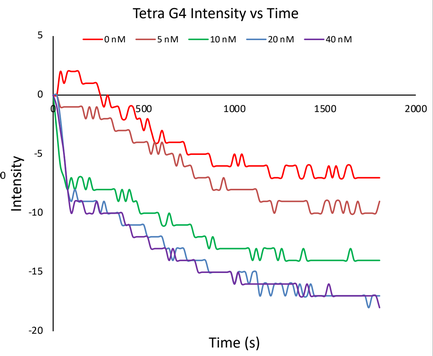

Figure 8: Graph of Intensity vs Time of the FRET Data for Tetra Molecular G-Quadruplex DNA Substrate with varying *Taq* UvrD concentrations of 0, 5, 10, 20, 40 nM.

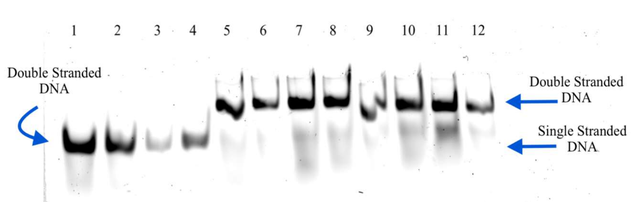

Figure 4: Helicase Assay Gel of Varying *Taq* UvrD Concentrations and Different DNA Substrates. Lanes 1-4 are Blunt End DNA with 5 nM, 10 nM, 20 nM and 40 nM enzyme. Lanes 5-8 are 53 base pair long overhang DNA with 5 nM, 10 nM, 20 nM and 40 nM enzyme. Lanes 9-12 are 43 base pair long overhang DNA with 5 nM, 10 nM, 20 nM and 40 nM enzyme.

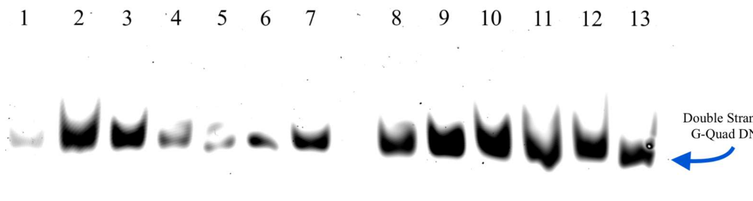

Figure 5: Helicase Assay Gel of Varying *Taq* UvrD Concentrations and Different DNA Substrates. Lanes 1-4 are Tetramolecular G Quadruplex DNA with 5 nM, 10 nM, 20 nM and 40 nM enzyme. Lanes 5-8 are Bimolecular G Quadruplex (Parallel) DNA with 5 nM, 10 nM, 20 nM and 40 nM enzyme. Lanes 9-12 are Bimolecular G Quadruplex (Anti) DNA with 5 nM, 10 nM, 20 nM and 40 nM enzyme. Lane 13 is Bimolecular G Quadruplex (Anti) DNA with no enzyme.

	E.Coli	Taq	TTE
Km (uM)	---	1500	45
Kcat (s^-1)	---	0.76	1.8
Vmax (nM/s)	---	20	92

Table 1: Calculated Kinetic Parameters of our enzymes: *E.Coli* UvrD, *Taq* UvrD, and TTE.

	% Unwound	
	43 overhang	53 overhang
Taq	57	46
TTE	62	65

Table 2: % Unwound Data for Taq at 10 nM and for TTE at 1.5 nM for 43 and 53 bp overhangs

Acknowledgements

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References

1.Brosh, J. . R. M. UvrD helicase: The little engine that could. *Cell Cycle* **13**, (2014).
2.Paul, T. *et al.* E. coli Rep helicase and RecA recombinase unwind G4 DNA and are important for resistance to G4-stabilizing ligands. *Nucleic Acids Res.* **48**, (2020).
3.Sauer, M. & Paeschke, K. G-quadruplex unwinding helicases and their function in vivo. *Biochem. Soc. Trans.* **45**, (2017).
4. Shukla, K., Thakur, R. S., Ganguli, D., Rao, D. N. & Nagaraju, G. Escherichia coli and Neisseria gonorrhoeae UvrD helicase unwinds G4 DNA structures. *Biochem. J.* **474**, (2017).