Helicase and ATPase Activity of *Thermus aquaticus* UvrD

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APPLIED PHYSICAL SCIENCES

Background

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- Mismatch repair is a vital cellular mechanism that repairs errors that occur during DNA replication or recombination.
- UvrD has been well characterized in *E. coli* as an ATP-dependent helicase[1] that is critical to prokaryotic mismatch repair (MMR)
  - Unwinds DNA unidirectionally in 3' to 5' direction[2]
  - Requires MutL and MutS accessory proteins to function[3]
- Eukaryotic UvrD is not well characterized
  - *T. aquaticus* UvrD will be used as hybrid system for this experiment
  - Similar to eukaryotes, *Taq* does not use methyl-directed DNA repair and lacks the accessory proteins that are necessary for prokaryotes

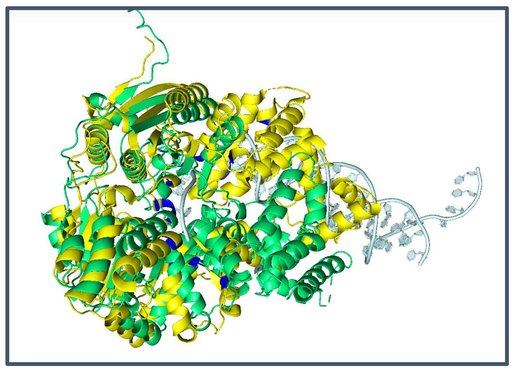


Figure 1. The structure of *Taq* UvrD (green) compared to *E.* coli homolog (yellow) and their interaction with the structure of dsDNA (generated using Pymol)

Methods

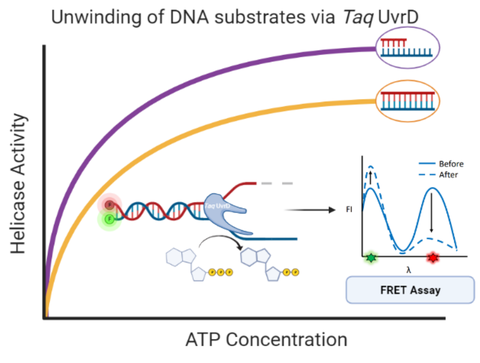
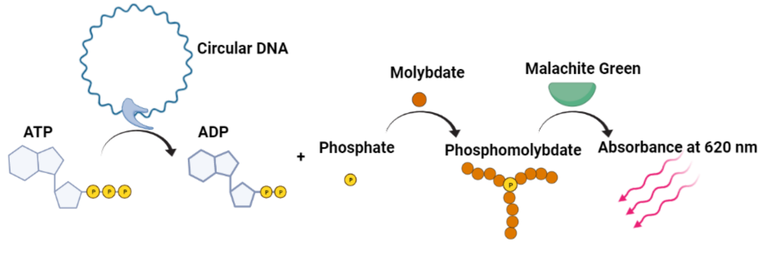
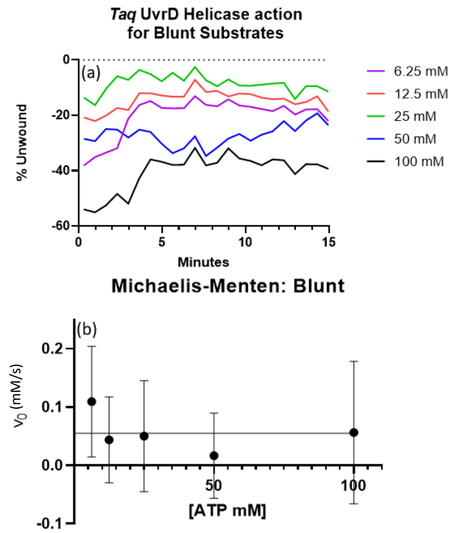


Figure 2. (left) Expected helicase activity of *Taq* UvrD in response to increasing ATP concentration was determined by FRET helicase assays

Figure 3. (below) ATPase activity of *Taq* UvrD is determined by measuring the free phosphate released by helicase activity



Helicase Activity



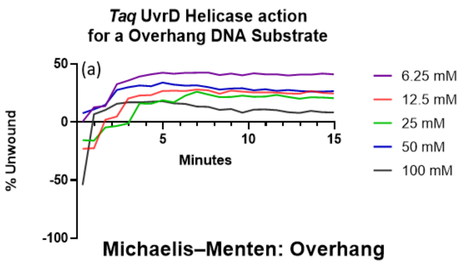
Blunt Substrate:

Figure 4. For the Blunt Substrate:(a) Calculated % Unwound DNA using both the emission plot and Eq. 1. (b) Michaelis-Menten plot generated by averaging the rate of unwinding over 3 minutes.

Parameters

K_m = 5x10^{-32} mM

v_{max} = 0.055 mM/s



3' Overhang Substrate:

Figure 5. For the 3' Overhang Substrate: (a) Calculated % Unwound DNA using both the emission plot and Eq. 1. (b) Michaelis-Menten plot generated by averaging the rate of unwinding over 3 minutes.

Parameters

K_m = 1.751 mM

v_{max} =0.233 mM/s

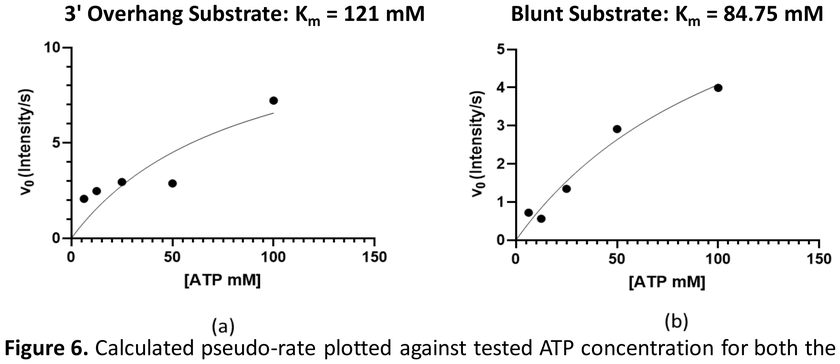
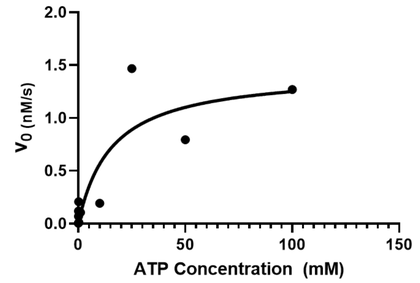


Figure 6. Calculated pseudo-rate plotted against tested ATP concentration for both the 3' overhang substrate (a) and blunt substrate (b). Fit Michaelis-Menten models reveal a K_m = 121 mM with a v_{max} = 9.01 intensity/s for the blunt, and a K_m = 84.75 mM with a v_{max} = 12.13 intensity/s for the overhang

ATPase Activity

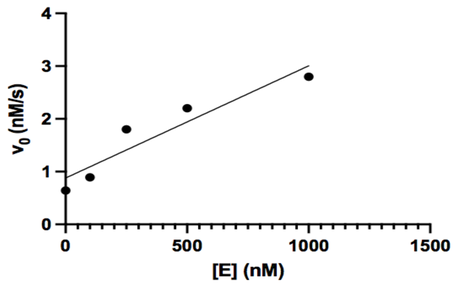


Parameters

v_{max} = 1.445 nM/s

K_m = 15.68 nM

Figure 7. The amount of ATP hydrolyzed by *Taq* UvrD showed hyperbolic dependence on the concentration of ATP. This fit revealed the enzyme had a K_m of 15.68 mM, a v_{max} of 1.445 nM/s



Parameters

k_{cat} = 3.12x10^{-3} s^{-1}

Specificity constant:
k_{cat}/K_m = 1.358 mM^{-1} s^{-1}

Figure 8. The amount of ATP hydrolyzed by *Taq* UvrD showed a linear dependence on the concentration of enzyme. This fit revealed the enzyme to have a k_{cat} of 3.12x10-3 s^{-1} and a specificity constant of 1.358 mM^{-1} s^{-1}

Conclusion, Significance, and Future Directions

Conclusion

Taq UvrD followed expected trends of enzyme kinetics, although its v_{max} and specificity constant indicate that it is quite slow and inefficient (Figures 7 and 8). *Taq* UvrD shows a better unwinding capability with the 3' overhang substrate than the blunt substrate (Figures 4 and 5).

Significance

These results provide more insight into the rate at which *Taq* UvrD catalyzes the unwinding of DNA to assist in MMR. A better understanding of an enzyme critical to the MMR mechanism offers a better understanding of how to treat diseases arising from failures in the MMR pathway.

Future Directions

Attempt to get working data from native gel assays to verify unwinding activity.

References

1. Erie, D. A. and K. R. Weninger (2014). "Single molecule studies of DNA mismatch repair." DNA repair **20**: 71-81.
2. Fukui, K. (2010). "DNA Mismatch Repair in Eukaryotes and Bacteria." Journal of Nucleic Acids **2010**: 260512.
3. Harfe, B. D. and S. Jinks-Robertson (2000). "DNA mismatch repair and genetic instability." Annual review of genetics **34**(1): 359-399.

Biorender used to generate methods schematics (Fig. 2 and 3)
Graphpad Prism was used to generate all graphs