Tte UvrD and Mismatch Repair Characterization

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Introduction

Mismatch repair pathways are invaluable systems in all cells that detect erroneous mutations and correct them. If MMR pathways in humans are defective, the likelihood of harmful conditions such as cancer increase significantly.

Despite their importance, little is known about the mechanism of MMR pathways in eukaryotes. To learn more about eukaryotic MMR pathways, we characterized an important eukaryotic MMR enzyme, Tte UvrD Helicase.

- Purification
- Quantification
- Characterization of helicase activity using the following assays:
  - ATPase malachite green assay
  - Native gel helicase assay
  - Fluorescence Resonance energy transfer (FRET)

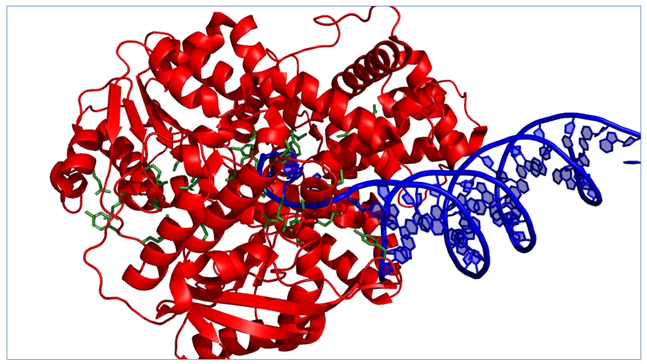
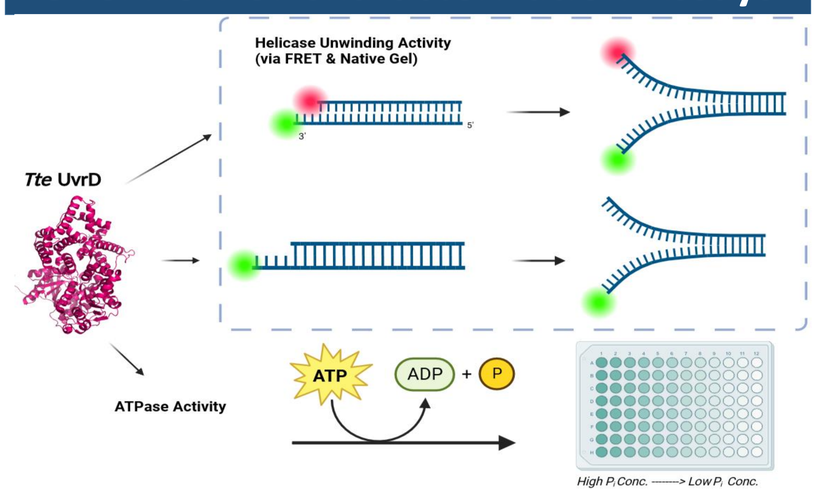


Figure 1: The structures of *T.te* UvrD (red) and DNA (blue) overlapped on one another. The binding sites between the two complexes are highlighted in green.

Overview of Characterization Assays



Results

ATPase Assay with Varying Protein Concentration

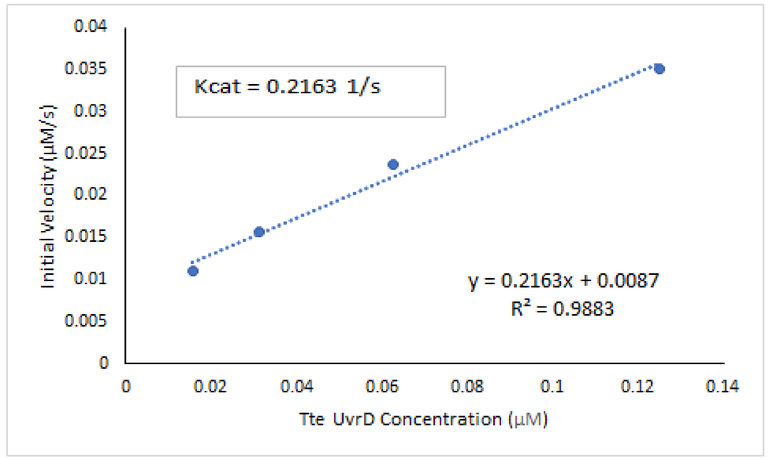


Figure 2: ATPase assay showing a positive correlation between amount of protein and organic phosphate concentration.

Native Gel Helicase Assay

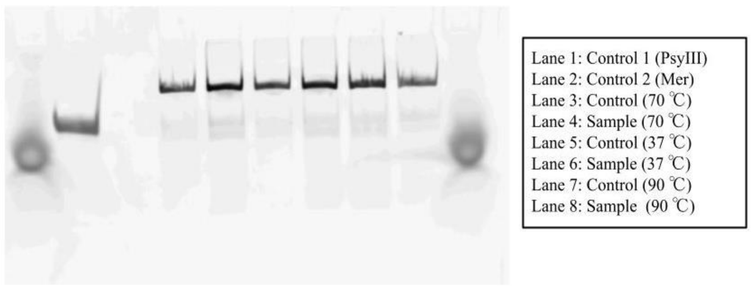


Figure 3: Native gel helicase assay testing unwinding activity of *Tte* UvrD at three different temperatures. Unclear if temperature affects unwinding.

FRET Assay with Varying ATP Concentration

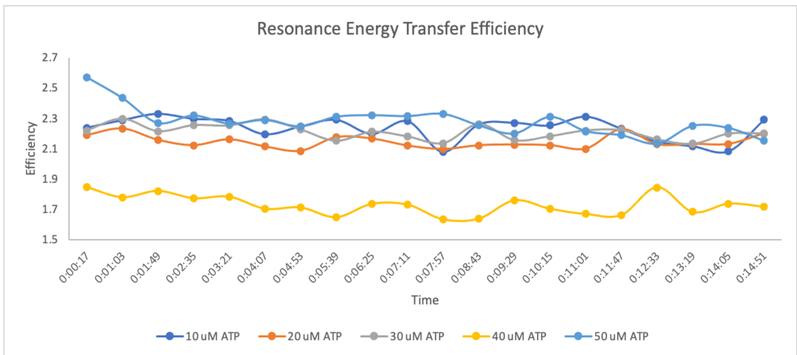


Figure 4: Inconclusive FRET assay showing no significant change in resonance energy transfer efficiency over a period of 15 minutes.

Conclusions

The native gel helicase assay did not provide conclusive results. The malachite green ATPase assay was most successful and provided information about the protein's catalytic activity. The data suggests that there is a positive correlation between the amount of protein and the hydrolysis of ATP. The FRET assays did not provide conclusive data.

Given the time and resources to further our research, more time would be spent researching similar helicases and the structure of UvrD helicases, and improving the techniques used to purify Taq UvrD.

Conclusions of hypotheses:

- UvrD unwinds duplexes with lagging strands more quickly than duplexes without lagging strands: *Inconclusive*
- As compared to E. coli UvrD, Tte UvrD acts to unwind all of the duplexes in a quicker manner at extreme temperatures as compared to the E. coli UvrD: *Inconclusive*
- When comparing the rate of unwinding of the G-quadruplexes, the biomolecular hairpin will unwind the fastest: *Inconclusive*

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References

- Li, G. M. Mechanisms and Function of DNA Mismatch Repair. *Cell Research* https://doi.org/10.1038/cr.2007.115
- Song, L.; Yuan, F.; Zhang, Y. Does a Helicase Activity Help Mismatch Repair in Eukaryotes? *IUBMB Life.* https://doi.org/10.1002/iub.349
- Kunkel TA, Erie DA. Eukaryotic Mismatch Repair in Relation to DNA Replication. Annu Rev Genet. 2015;49:291-313. doi: 10.1146/annurev-genet-112414-054722. PMID: 26436461; PMCID: PMC5439269.