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.

DNA Mismatch Repair (MMR) is a critical pathway that corrects mismatched base pairs that arise during DNA replication. 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.

The MMR helicase activity of eukaryotes is unknown and is hypothesized to be similar to MMR UvrD activity in prokaryotes. UvrD helicase functions to unwind DNA, and the UvrD helicase activity of Thermus aquaticus, which was selected because it is a homologous hybrid between prokaryotes and eukaryotes.

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

Characterizing UvrD Helicase in Thermus aquaticus

Emili Potts1, Holden Rogers1, Eyla Arteaga1, David Qiu1, Amy Aponte1, Thomas Freeman1, Ronit Freeman1
1Department of Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill, NC

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.

DNA Mismatch Repair (MMR) is a critical pathway that corrects mismatched base pairs that arise during DNA replication. 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.

The MMR helicase activity of eukaryotes is unknown and is hypothesized to be similar to MMR UvrD activity in prokaryotes. UvrD helicase functions to unwind DNA, and the UvrD helicase activity of Thermus aquaticus, which was selected because it is a homologous hybrid between prokaryotes and eukaryotes.

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

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: dG DNA, 43 base pair (bp) quadruplex (dnAP); blunt end dG DNA, 23 bp; blunt end dG DNA, 43 bp; parallel bi-molecular G-quadruplex DNA, 35 bp; and antiparallel bi-molecular G-quadruplex DNA. Data for each concentration of UvrD helicase was normalized and plotted against time.

B. Verification of UvrD Helicase Expression and Purification. From left most lane to right most: 1- Peptide-gel filtration protein ladder 2- 3% stock protein 3- 2% stock protein 4- Peptide-gel filtration 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: 43 base pair (bp) quadruplex (dnAP); blunt end dG DNA, 23 bp; blunt end dG DNA, 43 bp; parallel bi-molecular G-quadruplex DNA, 35 bp; and antiparallel bi-molecular G-quadruplex DNA. Data for each concentration of UvrD helicase was normalized and plotted against time.

D. Characterization of ATPase activity. Phosphate concentration calculated through measured absorbance and standard curve. Data normalized by subtracting substrate phosphate concentration calculated, from the sample containing G-DM of enzyme. 1. T. aquaticus UvrD ATP enzyme 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 calculated by dividing reaction time [20 minutes] from measured free phosphate concentration.

Results

A. Verification of UvrD Helicase Expression and Purification. From left most lane to right most: 1- Peptide-gel filtration protein ladder 2- 3% stock protein 3- 2% stock protein 4- Peptide-gel filtration 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.

B. T. aquaticus and T. tengcongensis UvrD helicase unwinding efficiency as a function of concentration. 1) dG DNA containing overhang lengths of 43 (circle) and 53 (diamond) nucleotides unwound with varied concentrations of T. aquaticus UvrD helicase. 2) dG DNA 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.

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.

Conclusions

Blunt end dG DNA, tetrameric G quadruplex DNA, parallel bi-molecular G quadruplex DNA, and anti-parallel bi-molecular G quadruplex DNA substrates were unable to be unwound by T. tengcongensis and T. aquaticus UvrD helicase.

The 43 and 53 base pair overhang dG DNA 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 conformational 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 mildly 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 proves the kinetic parameters of T. aquaticus helicase from being reported.

Acknowledgements

Equal contributions were made between group members. We would like to thank all 10A who contributed to the data collection process, as well as both Dr. Freeman for their organization efforts.

References


A. 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.