2023 CFID Undergraduate Summer Research Award Summary Report

Targeting Hepatitis B virus by pretending to be Hepatitis D virus

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Background

Hepatitis B Virus (HBV) is a leading cause of chronic liver disease, chronically infecting over 300 million people globally.[1-3] While an effective preventative vaccine exists, it does little for those already infected and current therapies can only suppress the virus. Therefore, there remains a strong drive and urgent need for research into unique and novel approaches to targeting HBV. Hepatitis D Virus (HDV) is a deficient RNA virus and cannot complete its replication cycle without support from the HBV surface proteins.[4,5] Therefore, HDV is only clinically found in those already infected with HBV. It is estimated that approximately 5% of individuals infected with HBV are co-infected with HDV (estimates range from approximately 12 million to 60 million people globally).[1-3] Clinically with HDV co-infection, HBV DNA levels appear to be quite low compared to HBV mono-infected individuals, which hints towards a potential viral competition scenario occurring between the two viruses.[6,7,8] The HDV genome essentially encodes for one protein, the small hepatitis D (SHD) antigen. The HDV genome also encodes a second protein, the large hepatitis D (LHD) antigen, only after its sequence is mutated, which extends the SHD by 19 amino acids.[9] Recently, it was discovered that these proteins (SHD and LHD) can bind the HBV pre-genomic RNA (pgRNA) and that the presence of the protein alone is sufficient to lower the levels of HBV DNA.[10] Specifically, the two proteins have been found to bind at the encapsidation signal (epsilon of HBV), interfering with HBV's ability to form new viral progeny.[10] How and exactly where the SHD/LHD bind the pgRNA epsilon segment is not yet known. Understanding these interactions at the molecular level will provide insight into an important viral competition dynamic and could lead to a novel therapeutic approach toward HBV.

Objectives

Overall, the objective of this research is to characterize the interaction between the SHD/LHD and HBV's pgRNA and to determine its structure at the molecular level for purposes of drug design. The two main aims are as follows:

Aim 1: To produce and purify HDV's SHD and LHD proteins.

Aim 2: To perform binding studies of the purified SHD and LHD with HBV's epsilon encapsidation signal to determine the binding affinities of SHD and LHD with the HBV epsilon region compared to positive and negative controls to determine a specific binding site.

During the duration of the CFID summer research project, the focus was on aim 1.

Methods

The SHD and LHD were recombinantly produced in *E. coli* BL21 cells using a *lac* operon bacterial expression system. SDS-PAGE was used to confirm the presence of soluble protein. The proteins were purified via affinity column chromatography using a glutathione-S-transferase tag. Next, the protein was further processed by cleavage of the tag and purified by size exclusion chromatography (SEC). Mass spectrometry was used to confirm protein identity. After obtaining purified protein, qPCR was run to determine protein functionality.

Protein production was optimized by testing different concentrations of isopropyl β-d-1-thiogalactopyranoside (IPTG) at different temperatures. Glycerol was also added to promote bacterial growth. The optical density obtained before starting induction was also optimized, along with the duration of time post IPTG addition before stopping induction.

The epsilon encapsidation signal was purchased through a commercial supplier, folded, and further purified by SEC. Fragments of HBV DNA downstream of the epsilon region was used as a negative control. Further controls used, included a segment of HDV RNA that is known to bind SHD (positive control) and a segment of HCV RNA (negative control).

The HBV pgRNA segment of interest was cross-referenced across 13,216 HBV reference sequences and a consensus sequence (at a variance of 0.1, 0.01, and 0.001) was determined using Geneious Prime. The consensus sequence was inserted into a pUC plasmid for in vitro transcription.

Progress and Next Steps

SHD was successfully produced and LHD production is currently being optimized. The optimal conditions for SHD production were determined to be with a concentration of 0.1M IPTG with 10M glycerol added. The seed culture was incubated for 16 hours at 22°C. The best yield was obtained after starting induction at an OD 600 between 0.6-0.8 and stopping induction after 2 hours.

SDS-PAGE confirmed the presence of soluble SHD protein. SDS-PAGE was also run after affinity column chromatography was used to purify the SHD protein-GST tag complex. Three bands appeared (at approximately 25kD, 30kD, and 35kD) which indicated a purer product. However, the bands appeared lower than expected. The band was expected around 50kD for the SHD-GST tag complex since the SHD protein is approximately 24kD in size (whereas the LHD is 27kD) and the GST-tag is approximately 25kD. The presence of three bands may indicate a break down product or the presence of a different protein so mass spectrometry was conducted to investigate this. The presence of SHD in the sample was confirmed via mass spectrometry. The GST tag was successfully removed via size exclusion chromatography, with a protein band appearing around 25kD in an SDS-PAGE gel, as expected.

We are now working on obtaining a larger yield of concentrated pure SHD protein, as well as growing and purifying the LHD protein. Once we have purified SHD, LHD, and HBV RNA segments, binding studies will be conducted as well as structural analysis, such as EMSA, MST, and SAXS.

To conclude, understanding the interplay between HDV and HBV may provide insight into novel and unique approaches to targeting HBV.

Personal Reflection

I am grateful to have received the CFID Undergraduate Summer Student Research Award. I was able to present my research at numerous conferences over the duration of my project, including the Biophysics Society of Canada Annual meeting (poster presentation), Infectious Disease Research Days (Flash Talk presentation), and ImmuNet Research Day (poster presentation). I also look forward to presenting my research at the upcoming AMMI conference! On top of receiving the opportunity to present my research, I have also enhanced my lab skills and learned a lot of new techniques that will help me in my future studies and professional endeavours.

References

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