Express and Purify the Capsid Protein (CP) of Human Astrovirus (HAstV) VP90$^{71-782}$ to uncover the structure of CP

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Astroviruses (AstVs): Star-Like Viruses

Astroviruses (AstVs)

- Positive-sense, single stranded RNA , nonenveloped viruses
- Having a characteristic star-like appearance in the center
- Factor in diseases ranging from diarrhea to encephalitis

Human Astroviruses (HAstVs)

- One of the main causes of gastroenteritis for infants and elderly people (Figure 2)

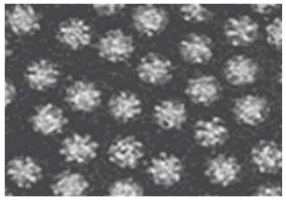

Image 1. Model of Astroviruses.
Adapted from [1]

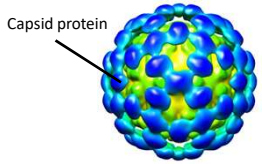

Figure 2. Model of Human Astroviruses.
Adapted from [2]

Role of Cleavage of Capsid Protein (CP) in Infectivity

- CP is a key to become infection
- CP interacts with the host cell during its entry and exit.

CP is synthesized as VP90.

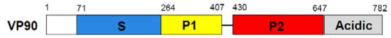

Figure 3. Structure of HAstV CP. VP90 Adapted from [3]

↓

VP90 undergoes proteolytic cleavage to VP70.

↓

VP70 is divided into three domains by another proteolytic cleavage.

↓

HAstV is mature and finally becomes infection.

Problem: CP Structure is unknown

- **Structure** of virus capsid is **unknown**.
- Revealing the structure of Capsid Protein will help **making vaccines** or **medicines** that can **prevent** the **infections**.

Project Objectives

Aim 1: Express and purify VP90$^{71-782}$ capsid particles

Aim 2: Make the crystals to characterize the structure by X-ray crystallization

Methods

Step1

- Culture bacteria which express the protein of VP90$^{71-782}$
- Purify the protein by Metal Affinity Chromatography
- Load SDS-PAGE to check the existence of the target protein

Step2

- Separate the protein by Size exclusion chromatography
- Load SDS-PAGE to check the existence of the protein

Step3

- Concentrate and screen the protein for crystal

Results

Step1

Purification by FPLC

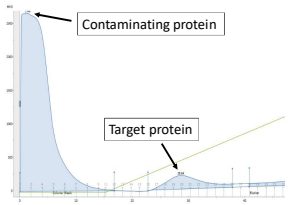

Figure 4: Purification of VP90$^{71-782}$.
VP90$^{71-782}$ was purified by metal affinity chromatography.

SDS-PAGE

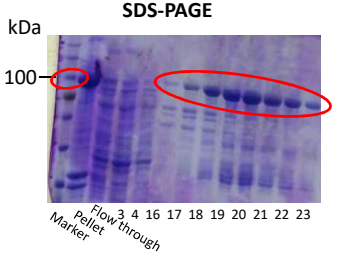

Figure 5: SDS-PAGE of VP90$^{71-782}$.
VP90$^{71-782}$ is around 100 kDa. This indicates that VP90$^{71-782}$ exists.

Step2

Purification by FPLC

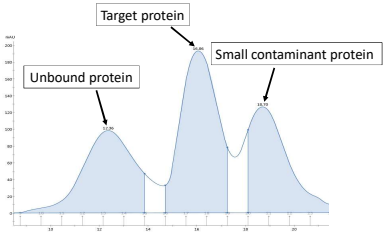

Figure 6: Purification of VP90$^{71-782}$.
VP90$^{71-782}$ was purified by size exclusion chromatography.

SDS-PAGE

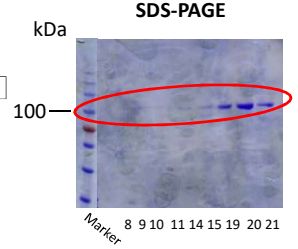

Figure 7: SDS-PAGE of VP90$^{71-782}$.
VP90$^{71-782}$ is around 100 kDa. This indicates that VP90$^{71-782}$ exists. However, the protein signal is low.

Step3

Crystallization

We are screening for crystal formation.

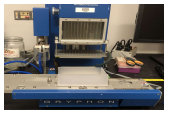

Image 8: Crystal Gryphon.
We used this for setting crystal plates.

Image 9: screening plate.
We screen this plate by optimal microscope.

Conclusion

- Nickle affinity purification was successful.
- Size exclusion purification was low protein concentration.
- The factor for having low protein signal is due to the protein being diluted during the second FPLC
- Revised procedure to increase protein yield and currently are running it.

Future Work

When We Get a Good Crystal

- Send the crystal to X-ray source
- Get the atomic structure of the protein

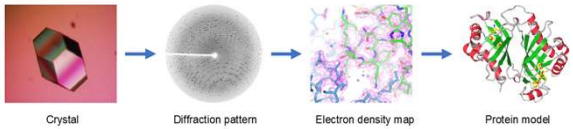

Figure 10: Workflow of X-ray crystallography.
X-ray crystallography can be used to determine the atomic structure of proteins.
Adopted from [4]

When We Get a High Molecular Weight Protein

- Prepare the protein by negative staining
- Observe the sample by using electron microscopy (EM)

If I could find a sample like Image 11,

- Observe the protein by cryo-EM (Figure 12)

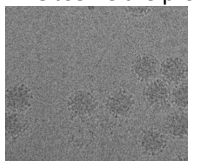

Image 11:
Image of immature HAstV-8.
This shows the sample of HAstV-8 Adopted from [5]

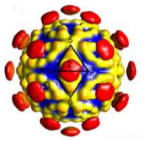

Figure 12: Cryo-EM structure of Mature HAstV-1.
This shows the sample of HAstV capsid.
Adopted from [3]

Acknowledgments

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Citations

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