Synthesizing Octahedral Polyomavirus Capsids for Cryogenic Electron Microscopy

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Introduction

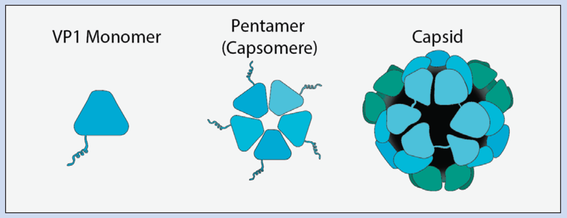


Figure 1. *Construction of capsids from individual VP1 monomers*

Polyomavirus capsids are polymorphic under different chemical conditions. These capsids have been synthesized artificially using purified VP1, the primary building block of the capsid. Five VP1 proteins form a highly stable pentamer, and these pentamers can form capsids (Fig 1). Depending on chemical conditions, the capsids can form three distinct sizes: T=7, octahedral and icosahedral (Fig 2). Only one form is found in infectious virions, and its structure has been well studied. However, no high-resolution structure of the octahedral capsid currently exists.

The goal of this research is to solve the structure of the octahedral capsid by electron microscopy and image classification. To do this, a streamlined protein purification protocol will be developed to isolate VP1 to provide many octahedral particles for successful electron microscopy.

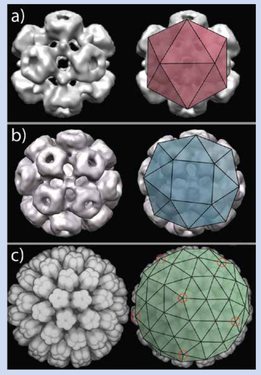
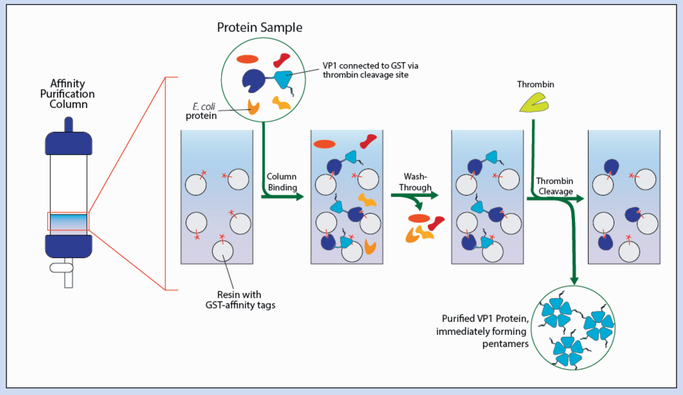


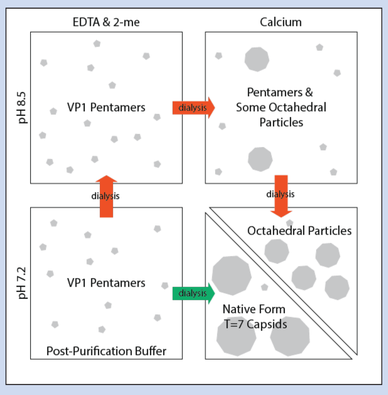
Figure 2. *Size differences in the three polymorphs of the Polyomavirus capsid: a) icosahedral, b) octahedral, and c) T=7*

Methods & Results

This research has included the development of this purification protocol:

- An expression vector containing GST-tagged VP1 obtained through a biotechnology company is inserted into E. coli cells.
- Cells are autoinduced to produce the target protein, then sonicated and centrifuged.
- Supernatant is placed in a glutathione column. On-column cleavage is performed (Fig 3). Further purified using ion exchange and size exclusion columns.
- Dialysis series is performed that has been shown to form octahedral particles (Fig 4).
- Imaged using Negative Stain Electron Microscopy (Fig 6).





Above: Figure 3. *VP1-specific protein purification protocol using glutathione resin and thrombin*
Left: Figure 4. *Pathway-dependent dialysis series for particle formation. Orange arrows are used to create octahedral particles.*

Experimentation has shown that autoinduction is optimized at 24 hours and thrombin cleavage at 12 hours. The washed beads with GST+VP1 bound show non-target binding (Fig 5), perhaps of *E.coli* proteins with glutathione affinity. Much of these contaminants are absent in the post-thrombin-cleavage elution, but traces of other involved proteins are in fact prominant.

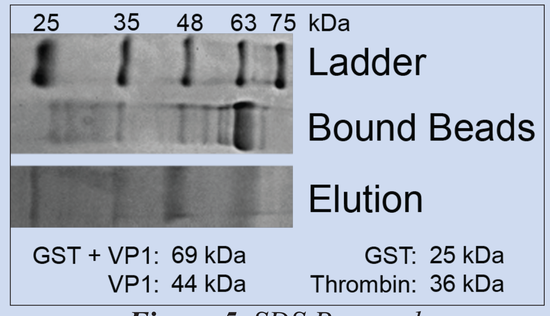


Figure 5. *SDS Page gel.*

The octahedral particle is ~32 nm in diameter. However, with negative stain, the measured size can be larger (35-39 nm).

The procedure has been successful in creating octahedral particles (Fig 6). However, the concentration and purity are not yet to the degree that Cryogenic Electron Microscopy requires to solve the capsid structure, so further purification is necessary.

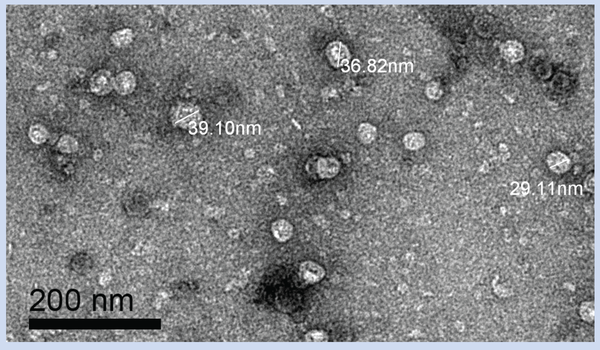


Figure 6. *Particles from most recent negative stain EM pictures*

Conclusion

The research is nearing the stage of Cryo EM, having made significant procedural adjustments that have streamlined and modernized protein purification. Yet to be identified is the concentration of salt that specifically targets the purification of VP1 pentamers in an ion exchange column. It is hypothesized that it the purity of the sample is the limiting factor that, once passed, will allow for effective particle creation.

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