Expression and Purification of the Human Astrovirus Capsid Protein VP90 for Structural and Functional Analysis

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One of the leading causes of gastroenteritis in children, the elderly, and the immunocompromised worldwide are the Human Astroviruses (HAstV). They are non-enveloped, T=3 icosahedral viruses with a positive, single-stranded RNA (+ssRNA) genome. The spread of the HAstV depends heavily on the consumption of contaminated food, with most healthy adults having antibodies against the common HAstV serotypes. However, studies indicate that emergent strains of HAstV can cause much more severe pathogenesis, including lethal encephalitis. There are currently no vaccines developed against HAstV, but there are ways to prevent viral infection. Viral entry can be inhibited by disrupting the capsid protein (CP) with antiviral compounds. The CP is encoded by the second open reading frame of the HAstV8 to form a 90kDa precursor capsid VP90 that undergoes proteolytic processing in order to mature. In order to understand how the HAstV assembles and matures, structural and functional analyses will be conducted on the VP90 capsid precursor. This work is focused on finding the optimal expression and purification conditions for VP90. Purified VP90 was isolated after expression with 0.3mM IPTG at 15°C induction temperature overnight and purifying with Ni-NTA column purification and size exclusion FPLC. A structural prediction of the VP90 particle was configured in order to visualize its capsid assembly. These VP90 particles will be used for EM imaging, crystallography studies, and biochemical analyses to better understand how HAstV becomes infectious.