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Short Communication

Atomic structure of a rationally engineered gene delivery vector, AAV2.5

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ABSTRACT

AAV2.5 represents the first structure-guided *in-silico* designed Adeno-associated virus (AAV) gene delivery vector. This engineered vector combined the receptor attachment properties of AAV serotype 2 (AAV2) with the muscle tropic properties of AAV1, and exhibited an antibody escape phenotype because of a modified antigenic epitope. To confirm the design, the structure of the vector was determined to a resolution of 2.78 Å using cryo-electron microscopy and image reconstruction. The structure of the major viral protein (VP), VP3, was ordered from residue 219 to 736, as reported for other AAV structures, and the five AAV2.5 residues exchanged from AAV2 to AAV1, Q263A, T265 (insertion), N706A, V709A, and T717N, were readily interpretable. Significantly, the surface loops containing these residues adopt the AAV1 conformation indicating the importance of amino acid residues in dictating VP structure.

1. Introduction

The Adeno-associated viruses (AAVs) are promising gene delivery vectors being developed for therapeutic applications. Current limitations to full realization of this system includes broad tissue tropism and neutralization by pre-existing host antibodies. Strategies to overcome these hurdles are focused on capsid sequence modification utilizing different approaches including error prone PCR, directed evolution, DNA shuffling, and structure-guided surface modifications by site-directed mutagenesis (Drouin and Agbandje-McKenna, 2013). The AAV2.5 chimera, combining features of parental AAV serotype 1 (AAV1) and AAV2, was generated using a structurally guided rational design strategy (Bowles et al., 2012). The vector was engineered with five AAV1 amino acids substituted into AAV2 based on comparison to muscle tropic serotypes AAV1, AAV7, AAV8, and AAV9 (Bowles et al., 2012). AAV2.5 was designed to bind heparan sulfate (HS), similar to AAV2, to display a muscle-tropic phenotype, like AAV1, and to have the ability to evade the immune response against AAV2 (Bowles et al., 2012). These phenotypes have been confirmed (Bowles et al., 2012). The vector was specifically designed to treat Duchenne muscular dystrophy (DMD), a monogenic disease affecting 1 in 5000 male births causing muscle degeneration, loss of mobility, and premature death (Gray et al., 2013; Iyombe-Engembe and Tremblay, 2017; Li et al., 2015; Mendell et al., 2010; Nelson et al., 2017, 2016; Ousterout et al., 2013; Wang et al., 2017). AAV2.5, packaging a miniaturized dystrophin gene, has been evaluated in a Phase I/II clinical trial with 5 pediatric patients and shown a promising safety profile along with gene expression (Bowles et al., 2012).

The AAVs package a single-stranded DNA genome of 4.7 Kb into a non-enveloped T = 1 capsid of ~260 Å in diameter. The sixty capsid viral proteins (VPs) assembling the capsids are the overlapping VP1, VP2, and VP3, in a predicted ratio of 1:1:10. The structures of numerous AAV serotypes, including AAV1 and AAV2, have been determined by either X-ray crystallography or cryo-electron microscopy and image reconstruction (cryo-EM), and show that only the VP3 common sequence is ordered. The capsid surface contains determinants of receptor attachment, tissue tropism, and antigenic reactivity, among other functions, within common variable regions (VRs) (Agbandje-McKenna and Kleinschmidt, 2011; Govindasamy et al., 2006; Halder et al., 2012). Here, the structure of AAV2.5 was determined to 2.78 Å using cryo-EM. The structure confirms the use of rational design to achieve desired capsid surface features without loss of the tertiary and quaternary structure to confer functional phenotypes.

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2. Materials and methods

2.1. Production and purification

The AAV2.5 CMV GFP vector was produced by triple transfection as previously reported (Bowles et al., 2012). Cell pellets were re-suspended in 20 mM Tris-HCl pH 7.4, 150 mM NaCl and 0.5% OPE (octylphenol ethoxylate, also known as Triton-X 100) as the lysis buffer and processed by a single pass through a microfluidizer then centrifuged at 2560 × *g* for 10 min to remove cellular debris. This partially clarified lysate was loaded onto a Heparin-6-fast flow column (GE) and washed with 20 mM Tris-HCl pH 7.4, 150 mM NaCl and 0.5% Triton-X 100 buffer followed by 1X PBS. The virus-containing fraction was eluted using 1X PBS with an additional 200 mM NaCl (to ~350 mM NaCl total). The eluted fraction was adjusted to 1 M NaCl (using a 5 M NaCl stock solution). This solution was loaded onto a hydrophobic interaction (Phenyl) column (GE), and the flow through fraction was collected. The 1 M NaCl flow through fraction was diluted to < 100 mM NaCl and subsequently loaded onto an SP Sepharose Fast Flow (SPFF) ion exchange column (GE). The column was washed with 1X PBS and the AAV2.5 vector was eluted with 1X PBS at 350 mM NaCl. The sample purity and integrity was monitored by 10% SDS-PAGE with Coomassie blue staining and electron microscopy (50,000 ×), respectively, and the concentration was determined using UV/visible light (Vis) spectrometry (260/280 nm; molar extinction coefficient, 1.7 for concentration in mg/ml).

2.2. Cryo-EM and data collection

Three microliters of AAV2.5 (~1.0 mg/ml) was applied to C-flat holey carbon grids (Protochips, Inc.), following glow discharge to increase hydrophilicity, and vitrified with a Vitrobot Mark IV (FEI Co.). The grids were screened with a Tecnai G2 F20-TWIN transmission electron microscope operated at 200 kV under low-dose conditions (~20 $e^-/Å^2$) prior to high resolution data collection. Cryo-EM movie frames were collected on a Titan Krios electron microscope (FEI Co.) operated at 300 kV with a DE20 direct electron detector (Direct Electron). Micrograph frame alignment was performed with the DE_process_frames software with the corresponding dark and bright reference images without radiation dose damage compensation (Spear et al., 2015). The data collection parameters are summarized in Table 1.

Table 1
Data collection parameters and statistics of final model.

Total number of micrographs	1592
Defocus range (μm)	0.6–4.0
Electron dose ($e^-/Å^2$)	59.34
No. of frames/micrograph	35
Pixel size (Å/pixel)	0.95
Starting no. of particles	30,773
No. of particles used for final map	24,618
Inverse B factor used for final map ($Å^2$)	1/100
Resolution of final map (Å)	2.78
PHENIX model refinement statistics	
Residue range	219–736
Map CC	0.871
RMSD (Å)	
Bonds	0.01
Angles	0.79
All-atom clash score	7.56
Ramachandran plot (%)	
Favored	98.26
Allowed	1.74
Outliers	0
Rotamers outliers	0
No. of Cβ deviations	0

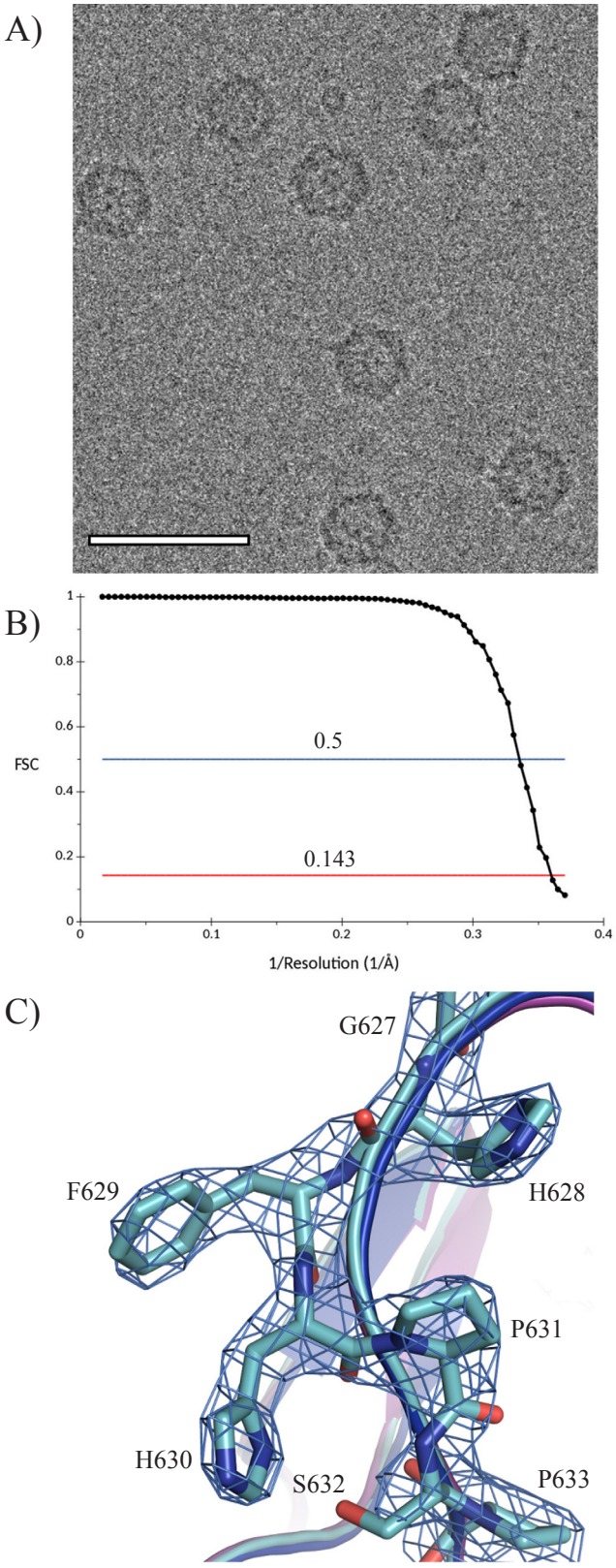

Fig. 1. Structure determination of AAV2.5. A) Cryo-electron micrograph of AAV2.5. Scale bar is 500 Å. B) Fourier Shell Correlation (FSC) plot from the last cycle of structure refinement. FSC 0.5 and FSC 0.143 are indicated. C) Piece of AAV2.5 electron density (blue mesh) showing residues 627–6333 to exemplify the quality of the map at 2.78 Å resolution. Residue types and number are as labeled. The main chain is also shown for AAV1 (purple), AAV2 (blue), and AAV2.5 (niobium).