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Structure of neurotropic adeno-associated virus AAVrh.8

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ABSTRACT

Adeno-associated virus rhesus isolate 8 (AAVrh.8) is a leading vector for the treatment of neurological diseases due to its efficient transduction of neuronal cells and reduced peripheral tissue tropism. Toward identification of the capsid determinants for these properties, the structure of AAVrh.8 was determined by X-ray crystallography to 3.5 Å resolution and compared to those of other AAV isolates. The capsid viral protein (VP) structure consists of an αA helix and an eight-stranded anti-parallel β-barrel core conserved in parvoviruses, and large insertion loop regions between the β-strands form the capsid surface topology. The AAVrh.8 capsid exhibits the surface topology conserved in all AAVs: depressions at the icosahedral twofold axis and surrounding the cylindrical channel at the fivefold axis, and three protrusions around the threefold axis. A structural comparison to serotypes AAV2, AAV8, and AAV9, to which AAVrh.8 shares ~84%, ~91%, and ~87% VP sequence identity, respectively, revealed differences in the surface loops known to affect receptor binding, transduction efficiency, and antigenicity. Consistent with this observation, biochemical assays showed that AAVrh.8 is unable to bind heparin and does not cross-react with conformational monoclonal antibodies and human donor serum directed against the other AAVs compared. This structure of AAVrh.8 thus identified capsid surface differences which can serve as template regions for rational design of vectors with enhanced transduction for specific tissues and escape pre-existing antibody recognition. These features are essential for the creation of an AAV vector toolkit that is amenable to personalized disease treatment.

1. Introduction

Viral vectors based on the non-pathogenic parvovirus adeno-associated virus (AAV) show great promise as gene delivery vectors due to their ability to deliver packaged foreign genes for stable and long-term protein expression in a wide range of human tissues (reviewed in (Flotte and Carter, 1995; Mingozzi and High, 2011)). They serve as the first approved gene therapy treatment (Salmon et al., 2014). These non-enveloped, single-stranded (ss) DNA packaging viruses belong to the genus *Dependoparvovirus* of the family *Parvoviridae* and require co-infection with a helper virus such as *Adenovirus* for replication (Bowles et al., 2006; Cotmore et al., 2014). Currently, clinical trials are underway with AAV vectors packaging therapeutic genes for the treatment of several diseases, including alpha1-antitrypsin deficiency, Leber's congenital amaurosis, muscular dystrophy, hemophilia B, cystic fibrosis, Alzheimer's disease, arthritis, lipoprotein lipase deficiency, Parkinson's disease, and HIV infection (e.g. (Carter, 2006; Coura Rdos and Nardi, 2007; Daya and Berns, 2008; Mueller and Flotte, 2008; Nathwani et al., 2011; Stieger et al., 2011)). Challenges for these clinical studies include the need to (I) improve viral-tissue specificity and (II) decrease the detrimental effects of the host immune response against the vector (especially for treatments that may require vector re-administration) (e.g. (Boutin et al., 2010; Michelfelder and Trepel, 2009; Tseng and Agbandje-McKenna, 2014)). In an effort to overcome these issues several novel AAV serotypes/variants have been isolated from nonhuman sources to exploit their varied tissue tropisms, transgene expression efficiencies, and expected lack of human immune system recognition.

To date, thirteen AAV serotypes (AAV1–13) and ~150 gene sequences have been isolated from human/non-human primate

Abbreviations: VP, viral protein; HS, heparan sulfate; VR, variable region; AAV, adeno-associated virus; ss, single-stranded; BBB, blood–brain barrier; EM, electron microscopy; NCS, non-crystallographic symmetry; NT, nucleotide; LamR, laminin receptor.

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tissues (Gao et al., 2004). Amino acid sequence comparison between these serotypes shows ~60–99% identity, with AAV4 and AAV5 being the most different (Gao et al., 2004). Of these 13 serotypes, AAV1, AAV2, AAV3, AAV5, AAV6, and AAV9 have human hosts (Atchison et al., 1965; Bantel-Schaal and zur Hausen, 1984; Gao et al., 2004; Georg-Fries et al., 1984; Hoggan et al., 1966; Melnick et al., 1965; Muramatsu et al., 1996; Rutledge et al., 1998), AAV4, AAV7, AAV8, AAV10, and AAV11 were isolated from nonhuman primates (Blacklow et al., 1968; Gao et al., 2002; Mori et al., 2004), and AAV12 and AAV13 were contaminants of Adenovirus stocks (Schmidt et al., 2008a,b). AAVrh.8, isolated from rhesus macaques, shares the highest sequence identity with AAV8 at 91% (Gao et al., 2004). Recent preclinical studies with AAVrh.8 have demonstrated that it efficiently transduces the retina, and crosses the blood–brain barrier (BBB) to transduce neuronal cells while also displaying reduced tropism for peripheral tissues (Giove et al., 2010; Yang et al., 2014). AAV9 and AAVrh.10 (another rhesus isolate) can also cross the BBB to target the central nervous system (CNS) (Foust et al., 2009; Hu et al., 2010; Yang et al., 2014; Zhang et al., 2011). Due to these properties AAVrh.8, AAVrh.10, and AAV9 are being actively developed for treatment of neurological disorders.

The AAVs package their linear ssDNA genome of ~4.7 kb into a $T = 1$ icosahedral capsid with a diameter of ~260 Å. The capsids are assembled from 60 copies (in total) of three viral proteins (VPs), VP1 (~87 kDa), VP2 (~73 kDa), and VP3 (~61 kDa) encoded from the *cap* gene and stochastically incorporated in an estimated ratio of 1:1:10, respectively (Buller and Rose, 1978; Johnson et al., 1971; Rose et al., 1971; Snijder et al., 2014). The VPs share a common C-terminal sequence (~520 amino acids; within VP3), with the entire sequence of VP3 contained within VP2, and all of VP2 contained within VP1, which has a unique N-terminal region of 137 amino acids (VP1u). Currently, structures of nine serotype members, AAV1–9, serving as the representative members of the AAV antigenic clades and clonal isolates, and AAVrh32.33, have been determined using X-ray crystallography and/or cryo-electron microscopy and image reconstruction (cryo-reconstruction) (DiMattia et al., 2012; Govindasamy et al., 2006, 2013; Kronenberg et al., 2001; Lerch et al., 2010; Mikals et al., 2014; Nam et al., 2007; Ng et al., 2010; Padron et al., 2005; Walters et al., 2004; Xie et al., 2011, 2002) (and unpublished data). Only the common VP3 region is observed in the structures. The conserved common core regions, which include a βA strand, an eight stranded β-barrel motif (βB-βI), and an α-helix (αA), are superposable while the apex of the loops inserted between the β-strands vary in sequence and structure. These regions are defined as variable regions (VRs) I–IX based on the comparison of two structurally diverse serotypes, AAV2 and AAV4 (Govindasamy et al., 2006). The VRs cluster at the icosahedral five-, three-, and twofold axes of symmetry to produce local variations on the capsid surface. Mutagenesis, biochemical, and structural studies demonstrate that residues in these VRs play important functional roles, including receptor attachment, transduction determination, and immunogenic reactivity (reviewed in (Adachi et al., 2014; Gurda et al., 2012, 2013; Kotchey et al., 2011; Li et al., 2008; Lochrie et al., 2006; Pulicherla et al., 2011; Raupp et al., 2012)).

In this study, the high resolution structure of AAVrh.8 was determined by X-ray crystallography to 3.5 Å resolution in an effort to pinpoint the capsid surface regions that differ in sequence and structure for this serotype and possibly responsible for its neuronal tropism. Similar to other AAV structures, only the VP3 common region of AAVrh.8 is ordered and conserves the VP topology. Comparison to selected AAV VP3 structures, AAV2, AAV8, and AAV9, identified the largest differences at VR-I and VR-IV with main-chain shifts of up to ~10 and ~5 Å, respectively, compared to AAV9. Minor main-chain shifts, for example up to 1.5 Å, were observed in the other VRs. However, significant differences were observed in some of the amino acid side-chain orientations even for conserved residues within these VRs. A heparin binding analysis showed lack of interaction for AAVrh.8 in contrast to the AAV2 positive control. In addition, native dot blot showed a lack of cross-reactivity with monoclonal antibodies (MAbs) and human donor serum directed against conformational AAV2, AAV8, and AAV9 epitopes. While these functional annotations are limited, the observations are consistent with published reports that residues within the VRs control receptor attachment and antigenicity for the AAVs. The AAVrh.8 structure thus identified surface loop regions to be (I) tested in an effort to pinpoint its neurotropism determinant, (II) modified in the development of AAVrh.8 and other AAV-based vectors for tissue-targeted gene therapy applications, and (III) modified in the development of vectors to evade pre-existing antibody responses.

2. Materials and methods

2.1. Virus production and purification

The recombinant vector used in this study, AAV2/rh8.TBG.GFP.BGH, was manufactured by PennVector at the University of Pennsylvania (Philadelphia, PA) as previously described (Wang et al., 2005). Briefly, a plasmid containing the enhanced green fluorescent protein (eGFP) transgene cDNA driven by the thyroxine binding globulin promoter and bovine growth hormone gene polyadenylation signal and flanked by AAV2 inverted terminal repeats, was packaged into the AAVrh.8 capsid by triple transfection using calcium phosphate precipitation. The helper construct used was the pAdΔF6 adenovirus helper plasmid. The recombinant rAAVrh.8-GFP vector was purified by three rounds of cesium chloride centrifugation and buffer-exchanged into phosphate-buffered saline (PBS). The vector titer (genome copies/ml (gc/ml)) was determined by real-time quantitative polymerase chain reaction (qPCR). For crystallization, the purified sample was concentrated to ~10 mg/ml using Apollo concentrators (Orbital Biosciences, MA) (150 kDa molecular-weight cutoff) by centrifugation at 2300×*g* at 277 K into PBS or Tris–HCl buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 6% glycerol (v/v)). The purity and integrity of the vectors was assessed by 12% SDS–PAGE with GelCode Blue staining (ThermoScientific, IL) and negative-stain electron microscopy (EM), respectively. For the EM visualization, samples were negatively stained with NanoW (Nanoprobes, NY) and viewed on an FEI Tecnai G2 Spirit electron microscope, as described in Halder et al. (2012b).

2.2. Crystallization, X-ray diffraction data collection, and data processing

Crystallization conditions were screened by varying the buffer (PBS or Tris–HCl), the concentration of polyethylene glycol 8000 (PEG 8000) (3–5.5% w/v), and NaCl (0.15–1.0 M). The crystal screens were set up using the hanging-drop vapor-diffusion method (McPherson, 1982) in VDX 24-well plates with siliconized cover slips (Hampton Research, Laguna Niguel, CA). Crystallization drops consisted of vector sample in the PBS or Tris–HCl buffer mixed with reservoir solution at a 1:1 ratio (v/v) and equilibrated against 1 ml of reservoir solution at RT.

Prior to data collection, the crystals were soaked for 30 s in cryoprotectant solution consisting of the reservoir solution and 30% glycerol (v/v) and flash-cooled in a liquid-nitrogen stream. A total of 302 X-ray diffraction images were collected from a single crystal at the F1 beamline at the Cornell High Energy Synchrotron Source (CHESS, Cornell University, Ithaca, NY) on an ADSC Quantum 270