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Identification of the heparin binding site on adeno-associated virus serotype 3B (AAV-3B)

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ABSTRACT

Adeno-associated virus is a promising vector for gene therapy. In the current study, the binding site on AAV serotype 3B for the heparan sulfate proteoglycan (HSPG) receptor has been characterized. X-ray diffraction identified a disaccharide binding site at the most positively charged region on the virus surface. The contributions of basic amino acids at this and other sites were characterized using site-directed mutagenesis. Both heparin and cell binding are correlated to positive charge at the disaccharide binding site, and transduction is significantly decreased in AAV-3B vectors mutated at this site to reduce heparin binding. While the receptor attachment sites of AAV-3B and AAV-2 are both in the general vicinity of the viral spikes, the exact amino acids that participate in electrostatic interactions are distinct. Diversity in the mechanisms of cell attachment by AAV serotypes will be an important consideration for the rational design of improved gene therapy vectors.

Adeno-associated virus (AAV) is a small (~25 nm) parvovirus, with an ~4.7 kb ssDNA genome packaged inside a non-enveloped capsid of T=1 icosahedral (60-fold) symmetry (Caspar and Klug, 1962; Xie et al., 2002). As a non-pathogenic virus, AAV has become a leading candidate vector for human gene therapy (Hildinger and Auricchio, 2004). Several naturally occurring serotypes of AAV have been identified, each having broad, but distinct tissue specificity (Buning et al., 2004; Mitchell et al., 2010). In addition to having broad tropism, AAV vectors are often neutralized in individuals previously exposed to virus or vector (Zaiss and Muruve, 2005). Knowledge of the structure and infectious pathway of AAV serotype capsids provides a template to engineer vectors that more specifically target diseased tissues, and to engineer neutralization escape variants that remain viable in cell entry (Buning et al., 2003; Flotte, 2004; Mitchell et al., 2010).

Prior to cell entry, AAV serotype 3 (AAV-3) attaches to target cells by binding heparan sulfate proteoglycan (HSPG) (Handa et al., 2000; Rabinowitz et al., 2002), but few details are known. In contrast, the binding site on AAV-2 for HSPG (or its analog heparin) has been well characterized, and is centered at Arg_{585} and Arg_{588} on the sides of the 3-fold proximal spikes (Kern et al., 2003; O'Donnell et al., 2009; Opie et al., 2003). Intriguingly, these residues are not conserved in AAV-3, and the determinants of receptor binding by AAV-3 remain unknown.

An understanding of the diversity in AAV-heparin interactions will advance our fundamental understanding of receptor attachment. AAV-3 is of particular interest because of its ability to transduce hematopoietic cells (Handa et al., 2000) and liver cancer cells (Glushakova et al., 2009) relatively efficiently. However, AAV-3 transduction levels are low for most cell types (Van Vliet et al., 2008). For AAV-2, heparin binding correlates closely with tissue specificity (Asokan et al., 2010; Grimm et al., 2008). In addition, the heparin binding site on AAV-2 can be replaced with peptide ligands to efficiently re-target vectors to desired tissues (Perabo et al., 2006; Shi and Bartlett, 2003; Shi et al., 2006). Similarly, detailed knowledge of receptor interactions by this serotype could increase its therapeutic potential.

We recently determined the crystal structure of AAV-3B (Lerch et al., 2010), a minor variant of AAV-3. The overall capsid structure is similar to that of other AAV serotypes which all have spike-like protrusions surrounding the 3-fold axes. Despite the structural similarity, the electrostatic surface potential of AAV-3B is quite different from that of other serotypes in the region corresponding to the AAV-2 HSPG-binding site. This has functional implications, as HSPG and heparin are negatively charged and typically form ionic interactions with basic regions on the surface of heparan-binding proteins (Conrad, 1998). Two regions near the spikes that are positively charged and unique to AAV-3B (Lerch et al., 2010), could, we hypothesized, facilitate receptor interactions in AAV-3B.

Abbreviations: HSPG, heparan sulfate proteoglycan; AAV, adeno-associated virus; SOS, sucrose octasulfate; NCS, non-crystallographic symmetry; WT, wild type; ELISA, enzyme-linked immunosorbant assay; GFP, green fluorescent protein.

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In the current study, interactions between AAV-3B and heparan sulfate analogs were investigated. The location of the receptor binding site was determined from crystallographic data from a complex of AAV-3B and an HSPG analog. AAV-3B capsid mutants were then used to (1) confirm the structural identification of the heparin binding site, (2) correlate heparin and cell binding to positive charge on the capsid surface, and (3) demonstrate the requirement of the heparin binding site for cellular transduction.

Results

Prediction of potential heparin binding residues

From the 2.6 Å crystal structure of AAV-3B (Lerch et al., 2010), candidate receptor binding sites were identified. Specifically, the electrostatic surface potential shows positively-charged regions near the 3-fold proximal spikes that are unique to AAV-3B (Fig. 1A). Heparin binding proteins typically interact with their ligands through one or a cluster of basic residues. One of the positively charged regions is centered on Arg_{447}. Arg_{447} is conserved in several other AAV serotypes, many of which do not bind heparin. In AAV-2, Arg_{447} is not involved in heparin interactions. In fact, its charge is neutralized by a salt-bridge with Glu_{499} (Fig. 1B). In AAV-3B, Glu_{499} is replaced by Asn_{500}, so the region is more positively charged. A second positively charged region is centered on Arg_{594}, the only surface-exposed basic amino acid in AAV-3B that is not conserved in other serotypes. Three Arg_{594} residues from adjacent subunits cluster at the 3-fold icosahedral axis to form the region of strongest positive charge on the AAV-3B surface (Fig. 1A).

Crystallographic identification of a receptor binding **locus** *on AAV-3B*

Diffraction data were collected from AAV-3B crystals grown in the presence of the heparin analog sucrose octasulfate (SOS). SOS has been used previously in structural studies of heparin-binding proteins (Innis and Hyvonen, 2003). The best crystal diffracted X-rays to 6.5 Å resolution and belongs to space group $F4_132$. Resolution of 6.5 Å is not sufficient to build atomic models, but it suffices to identify a ligand-binding site on a structure determined at 2.6 Å resolution, especially with the high quality maps available following 5-fold non-crystallographic symmetry averaging (Badger et al., 1988). The 2.6 Å native AAV-3B structure (Lerch et al., 2010) could be superimposed accurately on the SOS complex map by alignment of their icosahedral symmetry axes with no degrees of freedom (see Materials and methods). In fact, without any atomic refinement (which would be susceptible to over-fitting at 6.5 Å resolution), the native AAV-3B structure yields R^{cryst}/R^{free} of 0.28/0.27 when compared to the diffraction data of the SOS complex (Table 1).

A minimally biased maximum likelihood difference map, averaged according to the 5-fold non-crystallographic symmetry (NCS), was calculated using Fourier coefficients of $mF_o - DF_c$ and model phases, φ_c, where F_o and F_c are the observed and model structure amplitudes, m is the figure of merit weight, and D accounts for model errors (Read, 1986). A strong positive peak, 10σ above the mean density, signified something present in the crystals of SOS complex, but absent from the atomic model of uncomplexed virus. It was positioned between the spikes above the outer surface of the capsid. The density is centered on a viral 3-fold axis above the cluster of three Arg_{594} residues from neighboring capsid subunits (Fig. 2) that form the most positive region on the viral surface (see above). The peak is centered ~7 Å from the N_ω atoms of Arg_{594} and can accommodate one SOS molecule. Manually modeling with SOS places several sulfate groups within 3–5 Å of Arg_{594}, i.e. close enough for an ionic interaction. The density is located on a 3-fold symmetry axis, and therefore represents a mixture of 3 SOS orientations, precluding detailed modeling. Thus, corroborating experimental evidence would be sought (following sections) to characterize independently the functional significance of residues implicated by the low resolution crystallography.

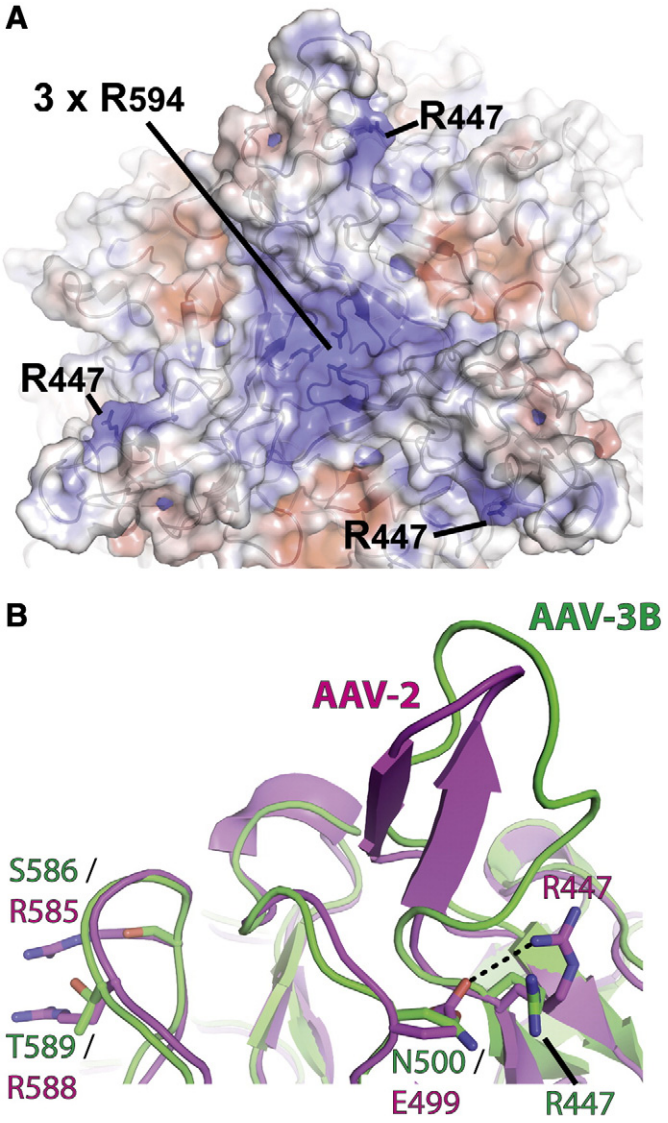

Fig. 1. Identification of potential heparin binding residues on AAV-3B. Two views near the 3-fold promixal spikes of AAV-3B are shown. (A) The spike-like protrusions of AAV-3B viewed down a 3-fold symmetry axis. A ribbon representation of the AAV-3B capsid can be seen beneath the translucent molecular surface, which is colored by electrostatic surface potential (blue = positive; red = negative). Two regions near residues Arg_{447} and Arg_{594} have strong positive surface charge and were identified as candidate receptor binding sites. (B) Structural overlay of a single spike from AAV-3B (green) and AAV-2 (magenta). Arg_{447} is conserved in AAV-2, but forms a salt bridge with Glu_{499} (dashed line). Asn_{500} is the equivalent residue in AAV-3B and, as a neutral amino acid, does not pair with Arg_{447}, leaving a stronger positive surface charge at this site. On the left, differences in the AAV-2 HSPG site are highlighted with Arg_{585} and Arg_{588} of AAV-2 replaced by Ser_{586} and Thr_{589} in AAV-3B.

Other features in the difference map were considered. A 10σ peak in the difference map near the tip of the spikes is unlikely to be SOS because: (1) it has an elongated shape, (2) modeling with SOS results in clashes with protein atoms, and (3) the contact surface is not positively charged. Locally high B-factors in all AAV structures indicate that the region is among the most disordered (Govindasamy et al., 2006; Lerch et al., 2010; Nam et al., 2007; Ng et al., 2010; Xie et al., 2002). The SOS complex and native AAV-3B crystal forms have distinct packing interactions at this exposed region of the surface that affect some, but not all of the NCS-related subunits, likely