P-10

Comparative Analysis of the Capsid Structures of AAV8, AAVrh.10, and AAVrh.39 and their Glycan Interactions

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The use of vectors based on Adeno-associated virus rhesus isolate 10 (AAVrh.10) for gene therapy applications has grown rapidly over the past few years. The advantages of AAVrh.10 include its high transduction efficiency in the CNS *in vivo* and the low percentage of pre-existing neutralizing antibodies in the human population. However, little is known about the basic biology of AAVrh.10. Here we present the AAVrh.10 capsid structure determined by cryo-electron microscopy (cryo-EM) and three-dimensional image reconstruction to 2.75 Å resolution. In parallel, the capsid structures of AAVrh.39 and AAV8 were also determined by cryo-EM to 3.27 Å and 3.10 Å resolution, respectively. In all the maps the side chain densities for most amino acids are well ordered allowing unbiased modeling of their VP3 and subsequent structure comparison. The capsid structure of AAVrh.10 is similar to AAVrh.39 and AAV8, with which it shares an amino acid sequence identity of 98% and 94%, respectively, resulting in an overall RMSD of 0.30 Å and 0.47 Å, respectively. The structures conserve the VP topology described for all AAV structures known to date. At the previously described AAV capsid surface variable regions (VR-I to VR-IX), only minor differences can be observed among the three viruses. Previously, AAVrh.10 was observed to interact with a sulfated LacNAc on a glycan microarray. Towards identifying the binding site on the capsid surface, the structure of AAVrh.10-LacNAc complex was determined by cryo-EM to 3.04 Å resolution. Additional density in the complex map, compared to AAVrh.10 alone, ordered between W505 and N472, was interpreted as bound galactose at the base of the 3-fold axis. This binding site corresponds to the galactose binding pocket in AAV9 and sialic acid binding site in AAV1 and AAV6. The implications of our structures and the interaction with the LacNAc glycan will be presented.