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Comparative Studies of AAV2 True Type and AAV2 Wild Type

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Adeno-associated viruses (AAVs) are ssDNA viruses utilized as gene transfer vectors in the treatment of genetic disorders. A new variant rationally engineered based on conserved natural AAV2 isolates, designated AAV2-True Type (AAV2-TT), is highly neurotropic compared to wild type AAV2 (AAV2-WT) *in vivo* and is being evaluated for CNS applications. AAV2-TT differs from AAV2-WT by 14 amino acids including R585S and R588T, the determinants of heparan sulphate binding by AAV2-WT. Here we present the comparison of AAV2-TT and AAV2-WT. The capsid structures of AAV2-TT and AAV2-WT were determined to 3.2 and 3.0 Å resolution, respectively, by cryo-electron microscopy and image reconstruction. The densities confirm the residue differences within the ordered VP3. The core β-strands, α-helix, and loop regions of both virus are superposable, with conformational differences observed at specific residues. These differences affect both inter and intra VP interactions. Differential scanning fluorimetry (DSF) used to analyze the stability of AAV2-TT and AAV2-WT at the pHs encountered during infection, 7.4, 6.0, 5.5, and 4.0, shows that AAV2-WT is ~5°C less stable than AAV2-TT at all the pHs tested, and maximal at pH 5.5. This pH corresponds to that of the late endosome and the point at which VP1u is proposed to become externalized to facilitate endosomal escape. This increased stability of the viruses may be important for maintaining capsid integrity while the viral genome is transported to the nucleus. Mutations converting AAV2-TT to AAV2-WT residues all appear to maintain the same capsid stability as AAV2-TT except residues S585R and T588R which caused a reduction in T_m. Similarly, the mutations of these basic residues in AAV2-WT R585S and R588T resulted in stabilization. These residues guide i*n vitro* cell binding and transduction, and affect the ability of the viruses to spread in the brain. Our comparative data will be presented.