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Top-down characterization of intact adeno-associated virus (AAV) capsid protein deamidation

Recombinant AAV (rAAV) vectors are a leading gene delivery system due to their low immunogenicity, tissue specificity, and high efficiency. Monitoring intact AAV capsid proteins (VPs) and their post-translational modifications (PTMs) is critical for ensuring product quality and efficacy. A crucial PTM to monitor is deamidation as it can alter product potency, stability, and function1. Reversed-phase intact LC-MS methods have proven effective in separating VPs from their deamidated counterparts2 while top-down MS approaches have been shown to be effective in characterizing individual proteoforms. Combining the two approaches can improve AAV VP characterization by allowing for detection of modifications on individual isoforms.

Commercial and in-house produced AAV products were used. Samples were analysed using a Vanquish Horizon ultra-high pressure liquid chromatograph hyphenated to an Orbitrap Eclipse mass spectrometer equipped with ETD, HCD, EThcD and UVPD fragmentation, as well as PTCR capabilities. Separation was performed using an ACQUITY Premier Protein BEH C4 (2.1 x 150 mm) column. Data was processed using Thermo Scientific Biopharma Finder software.

Along with the expected VP1, VP2, and VP3 proteins, deamidated proteoforms of each respective VP were separated. Unstressed and heat-stressed samples were analysed to examine whether changes in deamidation could be detected when samples were exposed to stressed conditions. Samples exposed to heat exhibited increased levels of deamidation, particularly in on VP3. A variety of different top-down (TD) fragmentation strategies were explored for characterizing the VPs of AAV9. Sequence coverage up to 5% was obtained by combining multiple fragmentation techniques. Additionally, 10% and 5% sequence coverage of VP2 and VP1 was achieved, respectively. The increased sequence coverage for VP3 is due to both its increased abundance and smaller size. Finally, thanks to the combination of multiple fragmentation techniques, TD-MS approach provided insights into the location of deamidation events, particularly for the stressed samples.

User consent

yes

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