A novel fragmentation-based multiple-attribute methodology (MAM) for AAV8 capsid protein analysis

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ABSTRACT

Adeno-associated virus (AAV) is one of the most commonly used delivery vehicles (vectors) in gene therapy development. It is a combination of proteins that form a closed shell (capsid) encompassing a single-stranded DNA called a transgene. The capsid consists of 3 viral proteins (VPs). The post-translational modifications (PTMs) of VPs can directly impact the viral infectivity and vector potency and are therefore considered critical quality attributes (CQAs) for gene therapy development. Here, an accurate mass LC-MS workflow is reported for analyzing AAV8 capsid proteins. A novel fragmentation type based on ExD was investigated using a comprehensive peptide mapping with multiple attribute methodology (MAM) focused on the relative quantification of PTMs. Three VPs were fully characterized with close to 100% sequence coverage. Multiple PTMs were identified with MS/MS confirmation. Some of them were found obtaining different levels in empty and full capsids.

INTRODUCTION

Recombinant AVV vectors are the most widely used vectors in gene therapy due to their low toxicity and longterm expression ability. AAV is a closed shell of proteins, called a capsid that encompasses a single stranded DNA of about 4.8 kD in size. The viral capsid of AAV8 is composed of 3 main proteins, which are 81 kD (VP1), 66 kD (VP2) and 59 kD (VP3) in size. VPs are critical for viral infectivity and vector potency. Thus, complete characterization of the constituent viral capsid proteins of AAV vectors, including their sequences and PTMs, is highly recommended to ensure AAV product quality and consistency. Herein, an accurate mass LC-MS-based peptide mapping with MAM workflow is reported for analyzing AAV8 empty and full capsids.

MATERIALS AND METHODS

Sample preparation:
Multiple lots of AAV8 samples were purchased from Vigene Biosciences. The titers were 5.10×10^{12} GC/mL–1.10×10^{13} GC/mL. Samples were concentrated with an Amicon Ultra filter (0.5 mL, 10 kDa MWCO) and then buffer exchanged to 25 mM of Tris, pH 8.0. The samples were denatured, reduced, alkylated and digested with trypsin, Lys-C and Asp-N with incubation at 37ºC overnight. The digest was subjected to LC-MS analysis (Figure 1).
HPLC conditions:
An ExionLC system (SCIEX) with a reversed-phase C18 column was used. The column oven temperature was set to 50ºC. The mobile phases were 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). 60-min linear gradient ramping from 1% to 40% B was applied for separation. The flow rate was 0.3 mL/min.
MS and MS/MS conditions:
A ZenoTOF 7600 system (SCIEX) was used for peptide mapping analysis with data-dependent acquisition mode, in which electron activated dissociation (EAD), a type of ExD, was used for MS/MS acquisition. Data were processed with Biologics Explorer software from SCIEX (Figure 2) for peptide mapping analysis followed by SCIEX OS software (SCIEX) for MAM processing.

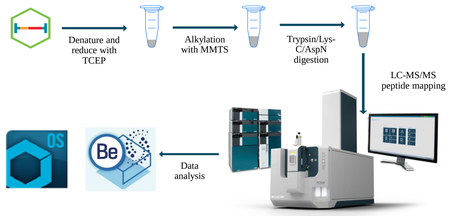

Figure 1. The general workflow for capsid protein peptide mapping analysis, including sample preparation, LC-MS data acquisition and data processing.

RESULTS

Three VPs—VP1, VP2 and VP3—were fully characterized with 100% sequence coverage at MS level and >95% sequence coverage at MS/MS level (Figure 3).

Multiple PTMs were identified with MS/MS confirmation. Representative MS/MS spectra of peptide with PTMs—including N-terminal acetylation, phosphorylation, deamidation and oxidation are shown in Figure 4. It is worth noting the identification and localization of the low-level PTMs (~0.1%) were achieved with solid MS/MS verification.

Selected PTMs went through the downstream MAM processing for full and empty AAV8 samples for proof-of-concept purposes. A batch processing in SCIEX OS software was executed to directly report the level of the PTMs (Figure 5). The levels were calculated as the peak area ratios between modified peptide and the sum of modified and unmodified peptides, based on the peak integrations of MS-level exacted ion chromatograms (XICs). Some PTMs were found obtaining different levels in empty and full capsids (Table 1).

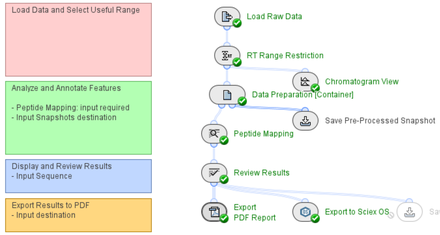

Figure 2. Overview of the peptide mapping workflow using Biologics Explorer software.

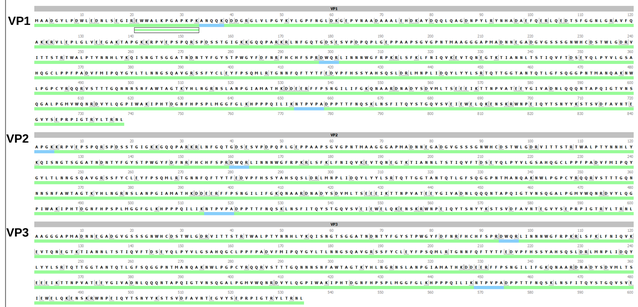

Figure 3. Sequence coverage map for VP1, VP2 and VP3. Green labeling represents the sequences that were verified with both MS and MS/MS, and blue labeling represents the sequences with MS verification.

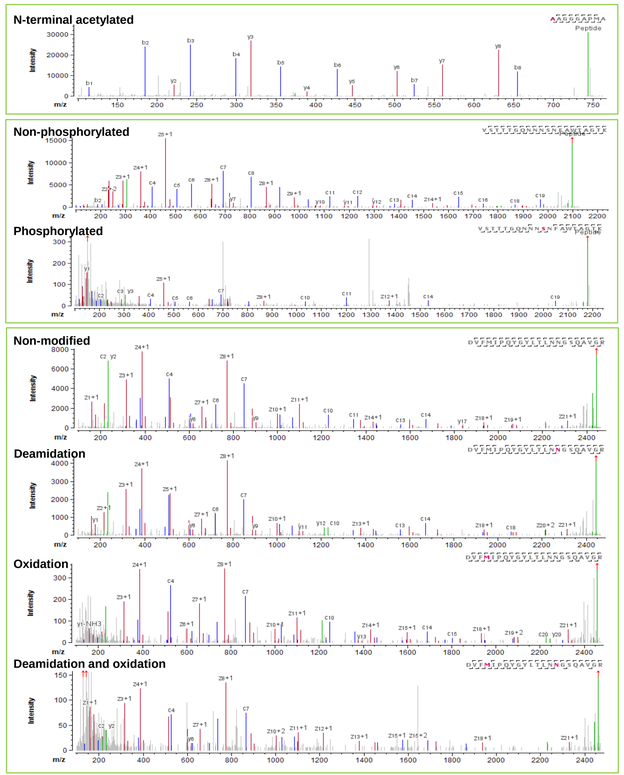

Figure 4. Representative EAD MS/MS spectra for AAV peptides. From top to bottom: (acetyl)**A**AGGGAPMA with N-terminal acetylation from VP3; VSTTTGQNNNS(phos)NFAWTAGTK from all VPs (including native and phosphorylated forms); DVF**M**(ox)IPQYGYLTLNN(dea)GSQAVGR from all VPs (including native, deamidation, oxidation, and deamidation + oxidation forms).

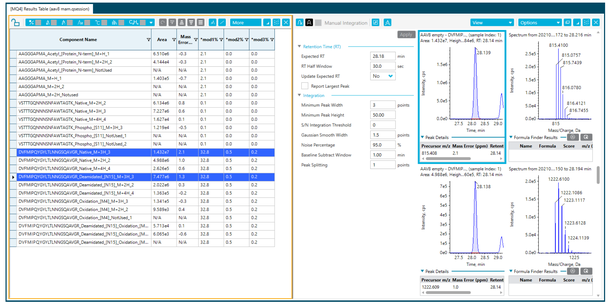

Figure 5. MAM processing user interface in SCIEX OS software. Left: result table with peptide and ion information, peak areas and calculated PTM%. Right: peak integration parameters, XICs and MS spectra.

Table 1. Levels of selected PTMs in empty and full capsids, calculated using SCIEX OS software processing.

Peptide sequences	Modification %	
	Empty	*Full*
(acetyl)**A**AGGGAPMA	2.1	4.7
VSTTTGQNNNS**(**phos)NFAWTAGTK	0.1	0.1
DVFMIPQYGYLTLNN**(**dea)GSQAVGR	32.8	26.9
DVF**M**(oxi)IPQYGYLTLNNGSQAVGR	0.5	0.6
DVF**M**(oxi)IPQYGYLTLNN**(**dea)GSQAVGR	0.2	0.2

CONCLUSIONS

EAD-based data-dependent analysis serves as an efficient workflow for in-depth peptide mapping combined with MAM in AAV8 capsid protein analysis.

REFERENCES

1 Xiaoying Jin, Lin Liu, Shelley Nass, Catherine O'Riordan, Eric Pastor and X. Kate Zhang. Direct Liquid Chromatography/Mass Spectrometry Analysis for Complete Characterization of Recombinant Adeno-Associated Virus Capsid Proteins. Human Gene Therapy Methods, 2017, 5, 255-267.
2 A new electron activated dissociation (EAD) approach for comprehensive glycopeptide analysis of therapeutic proteins. SCIEX technical note, RUO-MKT-02-12980-A.

TRADEMARKS/LICENSING