Characterization of proteins in influenza virus vaccines by LC-MS/MS and CGE

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ABSTRACT

This poster describes a method that employs mass spectrometry (MS) and capillary gel electrophoresis (CGE) to comprehensively characterize and quantify the pyrolysis virus vaccine to ensure vaccine quality and safety. Four proteins were identified by TOF MS after deglycosylation with PNGase F. The peptide map's sequence coverage of hemagglutinin (HA) and neuraminidase (NA) was close to 80%. The M459L mutation was confidently confirmed by the high-quality collision-induced dissociation (CID) data of 2 peptides. The detection of a signature y14 fragment allowed a confident assignment of 2 different sequences. CGE further confirmed these proteins in the influenza virus, consistent with the detection by LC-MS. The relative abundance of HA in the total protein was calculated.

INTRODUCTION

The major antigens of influenza viruses are HA and NA. Other proteins, including matrix protein (MP) and nucleoprotein (NP) are present at various degrees in vaccine production. While the efficacy of influenza vaccines is commonly monitored using the relative abundance of HA, both the structure and abundance of these proteins affect vaccine efficacy and safety.
There are many methods for the identification and purity characterization of HA protein, including reversed-phase high-performance liquid chromatography (RP-HPLC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and single radio immunodiffusion (SRID). However, the existing methods are insufficient due to low coverage or inaccuracy in identifying viral proteins.
The accurate molecular weight of the proteins were confirmed by LC-MS. Biologics Explorer software automatically identified sequences and confirmed mutations based on the MS and MS/MS data collected during the experiment. CGE was employed to achieve a more rapid and comprehensive assay for viral protein characterization and purity analysis, which enables high throughput and accuracy to evaluate the vaccine efficacy.

MATERIALS AND METHODS

Sample

1. MS with reduced sample: Influenza split vaccine B/Victoria was mixed with 7M guanidine Hydrochloride and 1M dithiothreitol and incubated at 52°C for 30 min.
2. MS with deglycosylated sample: Deglycosylated sample was created by mixing 20 µL of reduced sample with 4 µL N-glycosidase F, 4 µL phosphate buffer (pH 7.5), 4 µL Triton X-100 and 2 µL Tween 80 and then incubating the mix at 37°C for 1 h.

Desalting: Prior to analysis, all samples were desalted by 10 kD centrifugal filter.
3. CGE with non-reduced sample: For this analysis, 20 µL desalted influenza split vaccine B/Victoria were added to 70 µL sample buffer (100 mM Tris-HCl, 1% SDS, pH 9.0) and 5 µL 250mM iodoacetamide. It was then incubated at 70°C for 10min. For CGE-LIF, 1 µL P503 was added before incubating.
4. CGE with reduced sample: For this analysis, 20 µL desalted sample/deglycosylated sample was mixed with 70 µL sample buffer (pH9.0), 5 µL dithiothreitol and incubating at 70°C for 10min. For CGE-LIF, before incubating, added 1 µL P503.

LC-MS/MS

Analytes were separated using a Waters BEH200 SEC column (150 mm × 4.6 mm, 1.7 µm). Total method time was 10 min at a flow rate of 200 µL/min. Mobile phase was composed of 0.1% formic acid in 35% acetonitrile. Operating column temperature was 40 °C. Injection volume was 5 µL. LC-MS data were acquired and analyzed with a TOF MS method in SCIEX OS software using the X500B QTOF system.

CGE

CE-SDS experiments were performed using a PA 800 Plus system from SCIEX with a UV detector (using a 214 nm filter from SCIEX) and LIF detector (using a 488 nm rejection filter from SCIEX and a 600 nm emission filter from Edmund Optics). A prebuilt bare-fused silica capillary cartridge from SCIEX (PN A55625) with a length of 20/30 cm (effective length/total length) was also used. Approximately 90 µL of samples were loaded on sample vial trays for CE-SDS analysis. Data acquisition and analysis were performed using 32 Karat software (version 10) from SCIEX.

RESULTS

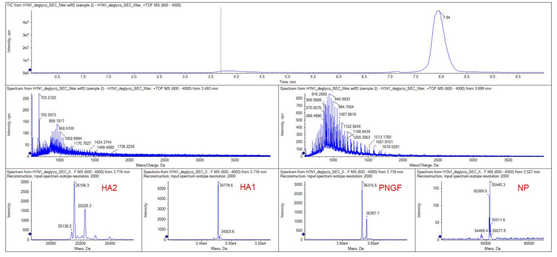

Figure 1. The raw MS spectra and deconvoluted spectra of the deglycosylated sample of the influenza split vaccine B/Victoria (BV). Four proteins with molecular weights of 25156.3, 34779.6, 36315.6 and 55440.3 were identified by primary mass spectrometry. According to the theoretical molecular weight of the proteins of BV virus, they were inferred to be HA2 deglycosylated protein, HA1 deglycosylated protein, PNGase F enzyme and NP protein, respectively.

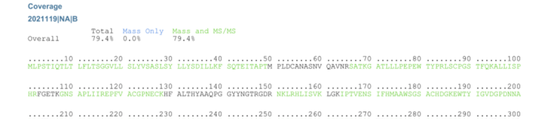

Figure 2. Sequence coverage for HA and NA peptide map from Biologics Explorer software processing. The HA and NA sequence coverage of the peptide map was nearly 80%. The low sequence coverage may be due to unknown mutations in HA and NA sequences of the influenza virus.

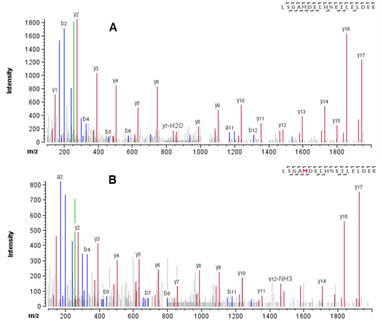

Figure 3. CID MS/MS spectra of the WT peptide and its M459L mutant.
The M459L mutation was confidently confirmed by the high-quality CID data of 2 peptides. The detection of a signature y14 fragment allowed confident assignment of 2 different sequences. Compared to Figure 3A, there was a series of mass changes from y14 ions to y17 in Figure 3B, which enabled increases confidence in the amino acid mutant analysis.

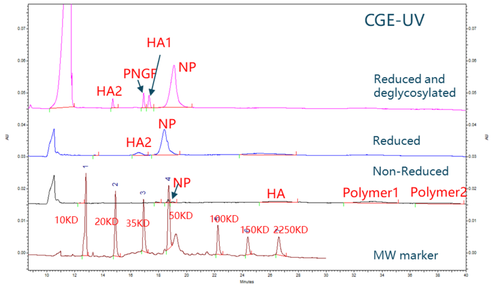

Figure 4. CGE-UV electrophoretogram of the influenza spilt vaccine B/Victoria (BV). The bottom panel shows the electrophoretogram of the molecular weight marker (MW marker). The next panel is the electrophoretogram of the non-reduced BV vaccine, followed by the electrophoretogram of the reduced BV vaccine. The top panel is the electrophoretogram of the reduced and deglycosylated BV vaccine. Peaks were confirmed by comparing their migration time to the migration time of the MW marker.
In the non-reduced electrophoretogram, the peak at around 18-20 min was inferred to be NP protein and 3 peaks after 25 min were determined to be HA protein and its polymers. In the reduced electrophoretogram, a peak at about 16.5 min was inferred to be HA2 and peaks at 18-20 min were inferred to be NP protein. For the reduced and deglycosylated electrophoretogram, the peak at 14.5 min was inferred to be the deglycosylated HA2, the 2 peaks at 17 min were inferred to be PNGase F and deglycosylated HA1 and the peak at 18-20 min was inferred to be NP protein.

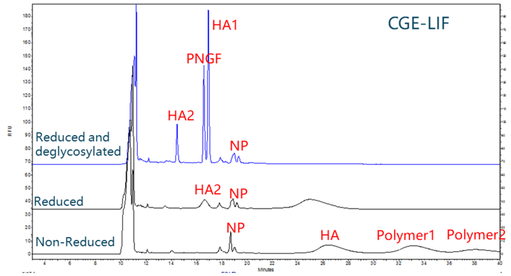

Figure 5. CGE-LIF electrophoretogram of the influenza spilt vaccine B/Victoria (BV). From bottom to top are the electrophoretogram for the non-reduced BV vaccine, the reduced BV vaccine and the reduced and deglycosylated BV vaccine". The separation results of CGE-LIF were consistent with those of CGE-UV.

CONCLUSIONS

- A rapid and accurate method was developed to evaluate the efficacy of influenza virus vaccines, including both characterization and quantitation
- The complex proteins in the influenza virus were isolated by CGE
- The CGE results of non-reduced, reduced and deglycosylated reduced samples were compared with each other, and the migration time of each protein was compared with MW marker to further confirm HA and NP proteins
- The HA, NA and NP proteins were identified by LC-MS/MS from primary mass spectrometry and sequence coverage, and the sequence coverage of HA and NA was about 80%

REFERENCES

1 E. van Tricht et al. (2015) New capillary gel electrophoresis method for fast and accurate identification and quantification of multiple viral proteins in influenza vaccines. *Talanta 144,1030–1035.*
2 Lars Geurink et al. (2021) Sixteen capillary electrophoresis applications for viral vaccine analysis. *Electrophoresis.* 0, 1–23.

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