Application of mass spectrometry-based proteomics in influenza virus research and vaccine manufacturing

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INTRODUCTION

Influenza virus is one of the most prevalent and highly contagious pathogens, which continues to have a significant impact on society. Despite the fact that this virus is thoroughly studied the disease is at best managed rather than controlled due to constant antigenic changes in two major viral coat proteins, haemagglutinin (HA) and neuraminidase (NA) that lead to the constant emergence of new virus strains against which there is sub-optimal immunity. The single radial immunodiffusion (SRID) assay is currently used to determine vaccine potency (Fig. 1). In order to perform SRID, HA must first be purified and then used for the production of HA-specific antiserum. This poses a potential bottleneck for vaccine manufacture and is of particular concern for the generation of SRID reference reagents and antisera for pandemic H5N1 and H1N1 vaccines. In addition, the test can be influenced by the physical state of the vaccine preparation (e.g. Tween-ether split vs. monovalent whole virus); it does not provide any information concerning presence of other viral proteins and possible non-viral protein contaminations; and it cannot distinguish between drift variants of different strains. Alternative proteomics-based assays that are independent of such reagents would alleviate those problems. This work reports on an ongoing effort to develop a mass-spectrometry (MS)-based technique that provides both qualitative and quantitative analysis of monovalent and trivalent influenza vaccine preparations and on development of an antibody-independent MS-based method that can determine the stability of HA in influenza vaccines without the need to generate or ensure stability of reagents (sheep antisera and reference antigens).

METHODS

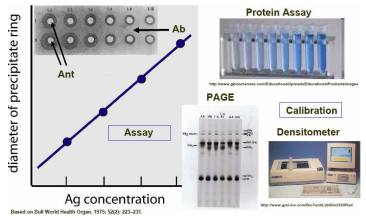
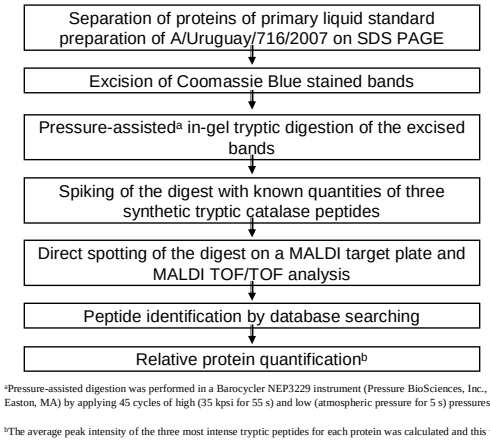


Figure 1: Scheme of Single Radial Immunodiffusion (SRID)

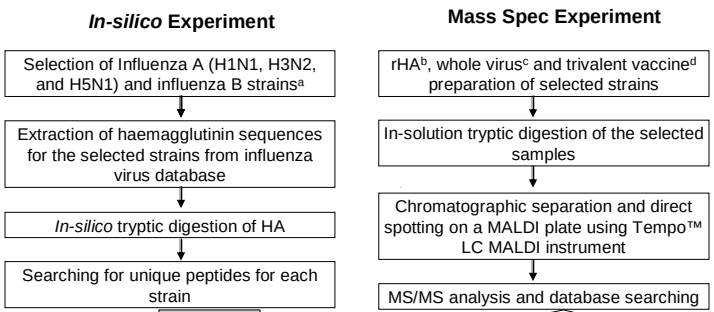


[a]Pressure-assisted digestion was performed in a Barocycler NEP3229 instrument (Pressure BioSciences, Inc., South Easton, MA) by applying 45 cycles of high (35 kpsi for 55 s) and low (atmospheric pressure for 5 s) pressures.

[b]The average peak intensity of the three most intense tryptic peptides for each protein was calculated and this value was normalized by the average peak intensity of the three synthetic tryptic peptides of catalase

Figure 3: Scheme of quantification of influenza virus proteins

METHODS



[a]The selected strains were: **Influenza A strain** (H1N1: A/Solomon Islands/3/2006, A/New Caledonia/20/99, and A/Beijing/262/95; H3N2: A/Brisbane/10/2007, A/Wisconsin/67/2005, A/Hiroshima/52/2005, A/New York/55/2004, A/California/7/2004, A/Wyoming/3/2003, A/Fujian/411/2002, A/Moscow/10/99, A/Panama/2007/99, and A/Sydney/5/97; H5N1: A/Turkey/15/2006, A/Indonesia/5/2005, A/Anhui/1/2005, and A/Vietnam/1203/2004) and **Influenza B strain** (Victoria-like: B/Ohio/1/2005, B/Malaysia/2506/2004, B/Hong Kong/330/2001, B/Shandong/7/97, and B/Victoria/2/87; Yamagata-like: B/Florida/02/2006, B/Sichuan/379/99, B/Johannesburg/5/99, and B/Yamagata/16/88

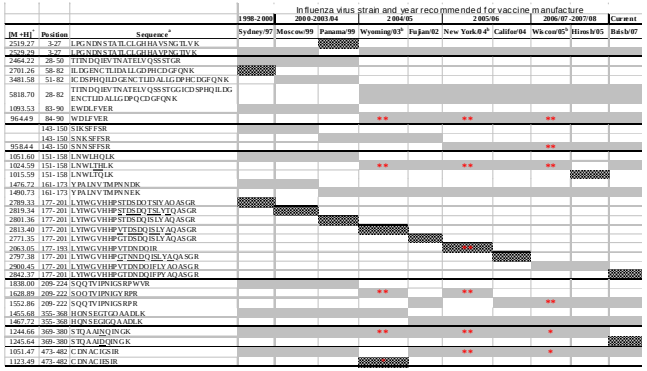
[b]Recombinant HAs from Influenza A virus (A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2), A/New York/55/2004 (H3N2), A/Wyoming/3/03 (H3N2), and A/Vietnam/1203/2004 (H5N1)) and Influenza B virus (B/Ohio/01/2005) produced in insect cells using the baculovirus expression vector system

[c]Whole virus preparation from Influenza A virus (A/New Caledonia/20/99 (H1N1) and A/Wisconsin/67/2005 (H3N2)) extracted from virus inoculated into 10-day old embryonated hen's eggs that were incubated at 33 °C for 3 days by ultracentrifugation

[d]The 2006/2007 Influenza Trivalent Vaccine containing A/New Caledonia/20/99, A/Wisconsin/67/2005, and B: B/Malaysia/2506/2004

Figure 2: Scheme of mass spec-based influenza virus strain identification

RESULTS



Diagonal crosshatch bars represent unique peptides to the specific strain. Gray bars represent *in silico* tryptic peptides expected in more than one strain.
[a] underlined amino acids represent sites of mutation.
[b] rHA from these strains were experimentally analyzed and peptides observed by MS/MS are marked by **, whereas those observed by MS only are marked by *.

Figure 4: *In silico* tryptic digest of HA from influenza A H3N2 strains used in vaccine preparation over the last 10 years

RESULTS

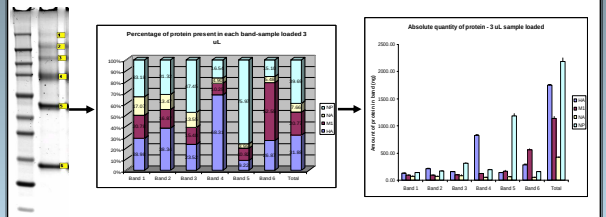


Figure 5: 1D SDS PAGE separation and quantification of influenza proteins from A/Uruguay/716/2007. Gel was run under non-reducing conditions.

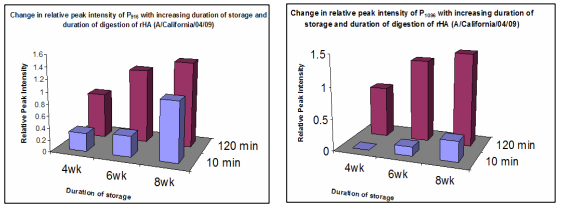


Figure 6: Mass spec-based stability study on rHA from A/California/04/09 stored in a refrigerator for 4, 6 or 8 weeks and digested with typsin for 10 or 120 minutes. Quantitative changes in relation to storage time and duration of digestion of two of the peptides, i.e., EFNHLEK (m/z = 916.4) and MNTQFTAVGK (m/z = 1096.5) are shown in the left and right panels, respectively.

CONCLUSIONS

- MS-based analysis of an influenza vaccine allowed rapid and accurate identification of each virus strain (Fig. 4). In addition, this approach enabled identification of other viral and host cell proteins coexisted in the viral preparation.
- 1DE separation of protein mixtures and tryptic digestion of excised bands followed by MALDI TOF/TOF analysis can be used as a label-free strategy for relative quantification of proteins (Fig. 5). We also confirmed the applicability of this technique in determining the relative quantities of proteins co-migrating in SDS PAGE of influenza virus preparations.
- Preliminary results of mass spec-based stability study on rHAs show this approach could have a potential to determine the stability of HA in influenza vaccines (Fig. 6).
- These methods have potential significance in the development of quality control assay for influenza vaccines and are relevant to the oversight of current influenza vaccines for several reasons:
  - ➢ Licensed inactivated vaccines are now available from five manufacturers and while the current methods ensure equivalent potency, there are no tests in place that confirm the HA strain or specifically identify proteins present in the final formulation.
  - ➢ At present influenza vaccines use virus that is grown in eggs, but it is expected that virus cultured in mammalian cells or recombinant antigens expressed in various cell types may be licensed in the future. Analysis of influenza vaccines by MS can be used to confirm inclusion of the appropriate HA and characterize vaccine composition more fully.
- Together with modern techniques to quantify the antigens and evaluate antigenic structure, these types of analysis can potentially be used for quality control of the final product that is distributed for human use.

"The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy."