Rapid Analysis of Intact Protein Using Liquid Chromatography Coupled with Mass Spectrometry

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ABSTRACT

Nearly every process conducted in a biotechnology company requires a tremendous amount of analytical characterization in support of the development of biopharmaceutical products. Because of the inherent complexity of bio-therapeutics, characterization of these large bio-molecules such as proteins and antibodies is significantly more challenging than it is for small molecules. Therefore, numerous instrumental methods are applied in order to fully characterize these high-molecular-weight products. Intact protein mass measurement becomes a routine practice for quality control and comparability studies during manufacturing changes. This research sought to develop a fast, robust workflow for accurate, highly sensitive intact protein mass measurements using liquid chromatography coupled with time-of-flight mass spectrometer. The workflow included an on-line trapping column packed with 10 µm poly (styrene-divinylbenzene) particles (POROS® R1) with 4000 angstrom sized pores for desalting and a column heater to increase mass transfer and diffusion. We showed examples of the MS measurements of antibodies as well as over-expressed human serum albumin. This method could easily be adapted to any core facilities that provide services regarding an intact protein MS measurement.

EXPERIMENTAL

MS instrument: AB SCIEX QSTAR XL quadrupole time of flight tandem mass spectrometer; Scan type: Positive scan mode “Enhanced All”; Electrospray: Nanoflow-LC ESI Source voltage: 2.30 kV; DP 100 V (increased DP imparts complex), DP2 45 V, CAD 6 V and FP 345V; Mass calibration: external; Data Processing: Bayesian protein reconstruct tool in BioAnalyst v1.1.5 software was used for deconvolution/transformation multiply-charged MS spectrum onto a true mass scale.

HPLC conditions: Waters CapLC; Column: Poros R1, 20 x 0.5 mm (trap) and 150 x 0.1 mm with PicoTip (analytical), 10 µm; Solvent A: Aqueous 0.1 % Formic Acid/3% Acetonitrile; Solvent B: 0.6% Formic Acid/20% isopropanol/75% Aetonitrile; Column heater: 50°C; Gradient: 0-6 min linearly programmed to 32% B, 6 min to 50% B, and 2 min to 85% B and hold 85 % B for 4 min. Inject volume: 50 µl; Total run time: 21 min.

RESULTS

DISCUSSION/ CONCLUSION

1. The Poros R1 is the ideal LC resin for intact protein analysis due to its intermediate hydrophobicity, and more importantly its wide 4000 Å pore size, which enables fast analyte diffusion. It is the resin of choice for LC separation of intact proteins.
2. Temperature of 50°C provided best chromatographic peak and the application of column heater on both trap and analytical columns helped sharpen the chromatographic peak (data not shown).
3. Higher sensitivity (low pmol on-column) is achievable in the system when a low flow rate is employed.
4. The method can also be used for top down proteomics research.
5. The method can be adapted for oligonucleotide analysis using R3 resin in negative electrospray mode.

Figure 3: Analysis of intact protein. The intact protein (about 12 pmol on column) was separated on the R1 column using a 14 min gradient. Top panel, LC peak; Middle panel, TOF MS spectrum; Bottom panel, reconstructed mass. A, apomyoglobin from equine heart; B, rice recombinant human serum albumin; C, IgG protein -PTG1.