Developments in On-Line Chromatographic Methods Coupled with Mass Spectrometry for the Characterization of Intact Proteins

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INTRODUCTION

LC-MS has become a powerful tool used in the characterization of complex samples. By far the most common mode of chromatography coupled with mass spectrometry has been reversed-phase. The coupling of reversed-phase chromatography with mass spectrometry has been a very effective tool in the characterization of protein pharmaceuticals. The most prominent use of this technique has been peptide mapping, where peptides obtained from proteolytic cleavage of the protein pharmaceutical are separated and identified. The advantage of this technique is the direct identification of peptides as well as modifications that might be present. Modifications such as oxidation, deamidation and glycosylation can all be determined using this technique. Here we show the use of other modes of chromatography coupled with mass spectrometry for the separation, identification, and characterization of intact proteins. Chromatographic separation modes based on size exclusion, ion exchange, reversed-phase, and affinity were evaluated. Each of these chromatographic separation modes coupled with mass spectrometry offers a different dimension in the characterization and separation of intact proteins.

METHODS and MATERIALS

System Components

Waters® BioSuite™ Intact Protein System
- Waters® 2796 Separations Module.
- Waters® 2487 Dual Wavelength Absorbance Detector
- Waters Mircomass® ZQ™ Mass Detector

Waters Micromass® Q-TOF micro™

Columns

Reversed-phase: BioSuite™ desalting cartridge, 2.1 x 10 mm
SEC: BioSuite™ 250, 5 µm HR SEC, 7.8 X 300 mm
Affinity: Prototype affinity column, 4.6 x 50 mm
IEC: Protein-Pak CM 8HR, 4.6 x 250 mm

Experimental Conditions

HPLC: Refer to Figures and Legends
MS: Source = ESI(+) Capillary (kV) = 3.3
Cone (V) = 25 and 30 (IgG1)
Temperature (°C) Source = 150 Desolvation = 425
Gas Flow (L/Hr) Cone = 50 Desolvation = 500
Scan Mode

Size Exclusion Chromatography (SEC)

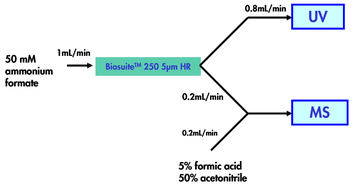

Figure 1: Schematic and experimental conditions for SEC coupled with mass spectrometry.

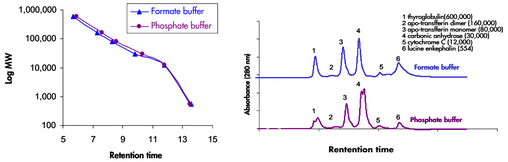

Figure 2: Comparison of traditional vs. MS compatible SEC buffers on a standard protein separation.

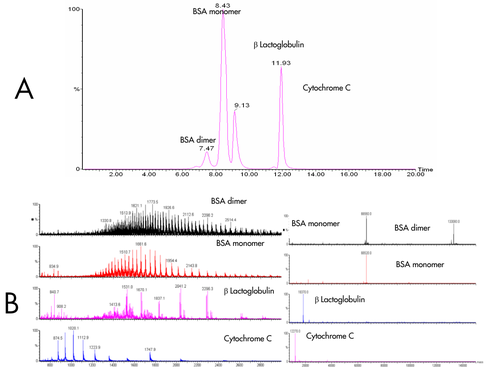

Figure 3A: Separation of a four component protein mixture by SEC-MS.

Figure 3B: Protein ion envelope and deconvoluted spectrum is shown for all proteins in the sample mixture, the addition of the MS enables for direct identification.

Affinity Chromatography

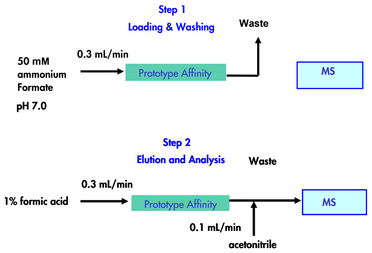

Figure 4: Schematic and experimental conditions for affinity chromatography coupled with mass spectrometry.

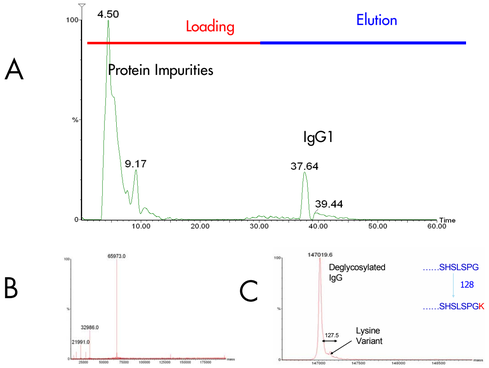

Figure 5A: On-line affinity-MS of crude ascites containing IgG1 is shown above a flow-through peak possible containing non IgG protein impurities can be seen at the retention time of 4.50 min. A smaller peak for IgG can also be seen eluting with 1% formic acid.

Figure 5B: Deconvolution of the major peak (4.50 min) shows a molecular weight of 65,973 which agrees well with the molecular weight of albumin.

Figure 5C: Deconvoluted spectra of deglycosylated IgG1. A peak for a lysine variant in IgG1 can also be seen.

Reversed-Phase Chromatography

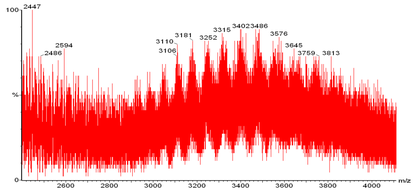

Figure 6: IgG1 and other proteins are typically stored in salt containing buffer. Salt suppress ionization of proteins during ESI-MS analysis. Salts also form adducts with proteins increasing the heterogeneity in the sample. Infusion of 20 picomoles of IgG1 in 20 mM Tris, 0.2% formic acid 50% acetonitrile is shown.

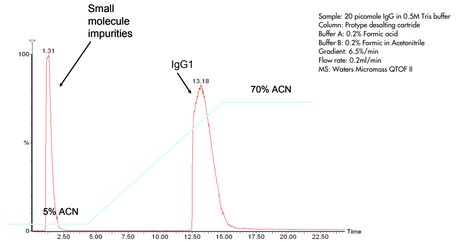

Figure 7: TIC showing separation of salts and other small molecule impurities from intact IgG1. The separation conditions are shown in upper right.

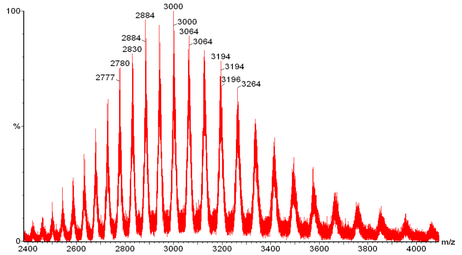

Figure 8: MS spectrum of IgG1 obtained after on-line desalting is shown. The amount of sample used (20 picomoles) is identical to that from Figure 6. A distinct enhancement in signal-to-noise ratio can be observed after online desalting.

Ion-Exchange Chromatography (IEC)

Gradient mobile phase conditions:
Tg= 25-50% B/15 min
A: 10 mM ammonium formate pH 5.0
B: 50 % formic acid

Figure 9: Schematic and experimental conditions for IEC coupled with mass spectrometry.

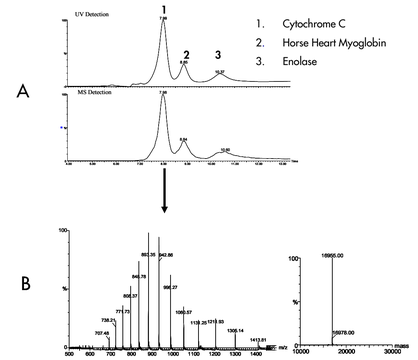

Figure 10A: Separation and detection of 10µg of protein mixture containing cytochrome c, horse heart myoglobin and enolase by IEC coupled with UV and MS detection.

Figure 10B: Protein ion envelope and deconvoluted spectrum confirming identity of peak 2 as horse heart myoglobin.

CONCLUSIONS

- IEC, SEC, and affinity chromatography can be directly coupled to mass spectrometry with mobile phase modifications.
- Different modes of chromatography can be successfully coupled with mass spectrometry for the identification and characterization of intact proteins.
- Affinity and reversed-phase chromatography where shown to isolate proteins from impurities that could interfere with analysis by mass spectrometry.