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

INTRODUCTION
IC-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 of reversed-phase chromatography with mass spectrometry 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 Micromass® ZQ™ Mass Detector
Waters Micromass® Q-TOP micro™

Columns
Reversed-phase: BioSuite™ desalting cartridge, 21 x 10 mm
SEC: BioSuite™ 250, 5 µm HR SEC, 7.8 x 300 mm
Affinity: Protein affinity column, 4.6 x 50 mm
IEC: Protein Pak CM BHR, 4.6 x 250 mm

Experimental Conditions
HPSEC. Refer to Figures and Legends.
MS: Source = ESI(n)
Capillary (HV) = 3.3
Cone (V) = 25 and 30 (5111)
Temperature (CF): Source = 150, Desolvation = 425
Gas Flow (L/H): Cone = 50; Desolvation = 500
Scan Mode

Waters®

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

CONCLUSIONS

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

Figure 5: On-line affinity-MALDI of circle octamer containing IgG1 is shown above a flow-through peak indicating obtaining 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 7: TIC showing separation of salts and other small molecule impurities from intact IgG1. The separation conditions are shown in upper right.

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

Figure 10A: Separation and detection of 105g of protein mixture containing cytochrome c, human hemoglobin and ovalbumin by IEC coupled with UV and MS detection.

Figure 10B: Proteolytic enzyme and deconvoluted spectrum confirming identity of peak 2 as human hemoglobin.

Figure 11: 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.

Figure 12: On-line IEC-MALDI of circle octamer containing IgG1 is shown above a flow-through peak indicating obtaining 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 13: Deconvoluted spectra of deglycosylated IgG1. A peak for a lysine variant in IgG1 can also be seen.