Capillary Electrophoresis-Mass Spectrometry (CE-MS) Analysis of Glycopeptides in Monoclonal Antibodies

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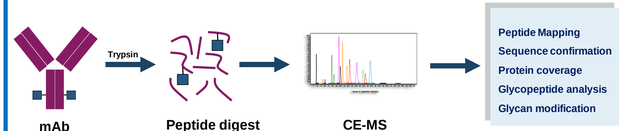
Background

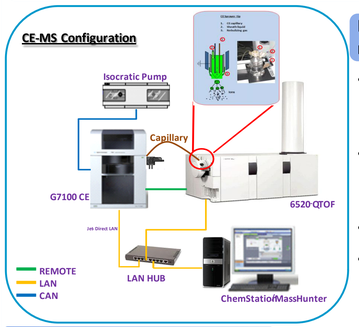
Glycosylation of monoclonal antibodies (mAb) can have impacts on it's biological activity and immunogenicity. Due to the importance of mAb as therapeutic agents, there is a growing demand for monitoring the carbohydrate structures attached to mAb. For enhanced separation efficiency, higher resolution, shorter run times, minimal sample/solvent consumption and flexibility, capillary electrophoresis (CE) has an enormous potential for the analysis of biopharmaceuticals. Further, there is growing interest in exploring CE coupled to mass spectrometry (MS) for the higher sensitivity and better compound identification with accurate mass measurements. Improvements in CE technology have made CE-MS a widely used tool for protein characterization. In the present work coupling of an Agilent 7100 CE system to an Agilent 6520 Accurate-Mass Q-TOF was achieved with a coaxial sheath liquid interface. The CE-MS setup equipped with electrospray source and orthogonal sprayer which reduces the risk contamination and improves the MS source cleanness. We have analyzed the glycopeptides of a mAb using this CE-MS setup. A tryptic digest of the mAb was subjected to CE-MS analysis and the glycopeptides were assigned using accurate mass measurement. Further, CE-MS/MS analysis was performed to search for diagnostic oxonium ions generated from a glycan moiety to identify the glycopeptides. The CE-MS platform, combined with the powerful data processing capabilities of Agilent MassHunter and BioConfirm software, enabled identification of the glycan modification attached to mAb complex.

Experimental



The mAb was lyophilized, reconstituted in ammonium bicarbonate containing TFE and DTT and then incubated at 95^{0}C for 20 min. To this solution, IAA was added and incubated at room temperature in the dark for 60 min. The solution was adjusted to pH 7-8 and trypsin digestion (20:1) was performed overnight incubating at 37°C. The samples were either immediately analyzed by CE-MS or stored at -20°C until use.

Instrumentation: CE-MS Setup



Recommendations for CE-MS measurements

- Adjust height of capillary inlet and MS spray tip to avoid siphoning effects
- Avoid or reduce non-volatile buffer components to increase sensitivity
- Optimize constant flow rates
- Use ground potential at outlet to simplify connection to ESI voltages

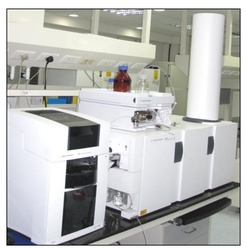
CE-MS sheath liquid interface

- Dual electrospray source and Orthogonal coaxial sheath liquid interface
- CE-MS interface via CE-MS adapter kit & sprayer Kit
- Sheath liquid through isocratic pump
  - equipped with a 1:100 flow splitter
  - mainly serves as electrical connect the CE outlet to ground potential at the sprayer
  - ensures application of maximum voltage across the capillary with stable electrospray

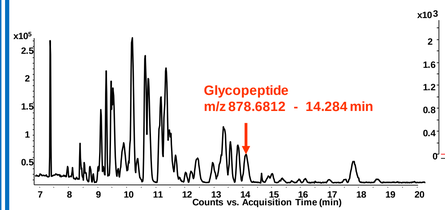
Equipments

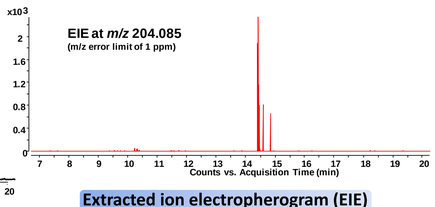
- Agilent Capillary Electrophoresis system (G7100)
- Agilent CE-MS Adapter Kit (G1603A)
- Agilent CE-ESI-MS Sprayer Kit (G1607A)
- Agilent 1200 series isocratic HPLC pump (automated sheath liquid delivery)
- Agilent 6520 Accurate-Mass Q-TOF with API Dual Electrospray Source
- Agilent ChemStation and MassHunter software packages

CE-MS conditions	
Capillary Electrophoresis	
CE:	7100 CE
Sample:	mAb digest
Injection:	10s @50 mbar (~ 0.4pmoles)
Capillary:	PVA, total length 60 cm, 50 µm ID
Buffer:	2% acetic acid
Voltage:	27 kV
Extr. pressure:	10mbar
Temperature:	20°C
Mass Spectrometry	
MS:	6520 Q-TOF
Ionization/Acquisition:	Dual ESI, 300-3200 m/z
Sheath liquid:	0.5 % acetic acid in 50 % MeOH
Sheath liquid flow:	4 µL/min
Drying gas flow:	5 L/min
Nebulizer:	10 psi
Drying gas temp:	150 °C
Fragmentor:	175 V
Vcap:	3500 V
Accu time:	333.3 ms/spectrum
Accu rate:	3 spectra/s



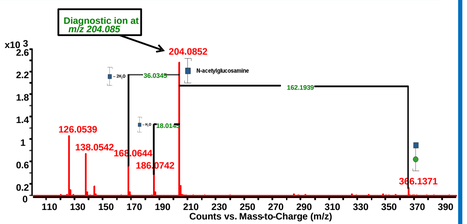
Results and Discussion





Extracted ion electropherogram (EIE)

Presence of glycopeptide was confirmed with intense diagnostic sugar oxonium fragment ions

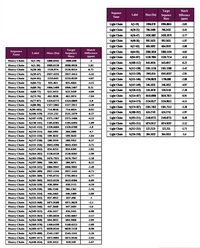


Base peak electropherogram (BPE)

Electrophoretic resolution of a BPE of **trypsin digested mAb**

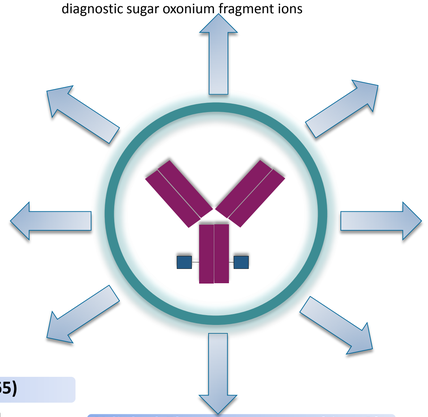
CE-MS/MS of Glycopeptide

Product ion spectrum of EEQYNSTYR with the assigned fragment structures

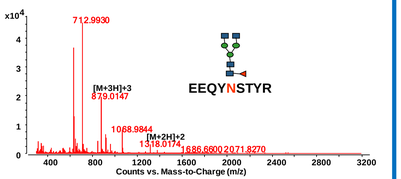


Peptide Mapping

The peptide masses obtained for the light and heavy chains were then matched with the theoretical digest using a 10 ppm error.

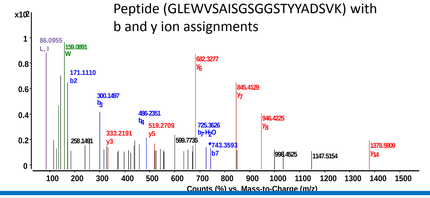


CE-MS spectrum of the glycopeptide



MS/MS spectrum of peptide (44-65)

Peptide (GLEWVSAISGSGGSTYYADSVK) with b and y ion assignments



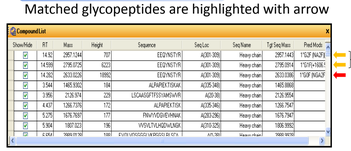
N-linked Glycans present on this mAb

Code	Oligosaccharide structure	Average Mass	Comments
G0F		1446.35	Major form found in this mAb
G1F		1607.49	Minor form found in this mAb
G2F		1769.64	Small amount found in this mAb

Galactose Mannose Fucose N-acetylglucosamine

MassHunter BioConfirm

Matched glycopeptides are highlighted with arrow



Conclusions

- CE-MS demonstration of rapid and sensitive characterization of an mAb peptides at low pmole levels.
- CE-MS/MS confirmation of a glycan moiety was made on the basis of the *m/z* 204 fragment.
- The flexible CE technology in combination with a Q-TOF is a valuable tool for studying glycoproteins.
- The combination of CE with Q-TOF MS is a valuable tool for peptide mapping of small quantity biopharmaceuticals.

References

- An Integrated Solution for CE-ESI-MS, Agilent Technologies publication number 12-5968-1328E
- Maria Serwe, Christine Miller, Analysis of peptides using CE/MS/MS, Agilent Technologies publication number 5988-1426EN
- Ravindra Gudihal and Keith Waddell, Glycopeptide and glycan analysis of monoclonal antibodies using a microfluidic-based HPLC-Chip coupled to an Agilent Accurate-Mass Q-TOF LC/MS, Agilent Technologies publication number 5990-5190EN