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 its biological activity and immunogenicity. Due to the importance of mAb as therapeutic agents, there is a growing demand for monitoring the carbohydrate structures of the mAb complex.

Experimental
The mAb was lyophilized, reconstituted in ammonium bicarbonate containing TFE, and DTT and then incubated at 95°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 7.4 and trypsin digestion (1:4) was performed over night incubating at 37°C. The samples were either immediately analyzed by CE-MS or stored at -20°C until use.

Results and Discussion
• CE-MS demonstration of rapid and sensitive characterization of an mAb peptides at low sample 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 glycosylated biopharmaceuticals.

Conclusions

Instrumentation: CE-MS Setup

CE-MS Configuration

• Dual electrospray source and Orthogonal coaxial sheath liquid interface
• CE-MS sheath liquid interface
• Agilent 1200 series isocratic HPLC pump (automated sheath liquid delivery)
• Agilent Capillary Electrophoresis system (G7100)

Equipment
• Agilent Capillary Electrophoresis system (G7100)
• Agilent CE-MS Adapter Kit (G1603A)
• Agilent CE-MS/MS Adapter 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

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