Characterization of Monoclonal Antibodies Using Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry (CE-ESI-MS)

Application Note

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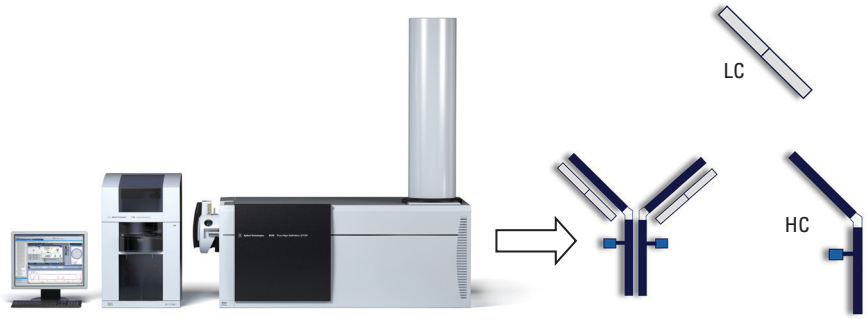
Abstract

This Application Note demonstrates the utility of capillary electrophoresis/mass spectrometry (CE/MS) for the analysis of intact monoclonal antibodies (mAbs). The Agilent 7100 CE System coupled to an Agilent 6530 Q-TOF LC/MS was used to study the intact and reduced forms of mAbs. The combination of mass measurements obtained by CE/MS and the data processing capabilities of Agilent MassHunter and BioConfirm Software enables the identification of heterogeneity in an intact mAb and its fragments. The result demonstrates the utility of CE/MS as a complementary technique to liquid chromatography/mass spectrometry (LC/MS) for the analysis of mAbs.



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Introduction

Therapeutic proteins such as monoclonal antibody (mAb) are gaining much attention in the biopharmaceutical industry. mAbs are susceptible to chemical modification and degradation during production, formulation, and storage. Hence, it is important to monitor the manufacturing consistency, purity, and molecular weight. Capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) with UV and fluorescence detection is a technique routinely used for quality control testing of mAbs[1]. However, there is growing interest in exploring CE coupled to mass spectrometry (MS) for higher sensitivity and better compound identification with accurate mass measurements.

The reagents and sample preparation procedures used for CE-SDS separation are not compatible with MS (mainly due to ion suppression issues). In addition, the assignment of peaks corresponding to post-translational modification is challenging in SDS-protein capillary electrophoresis. Although liquid chromatography/mass spectrometry (LC/MS) can be regarded as the gold standard for recombinant protein characterization, CE/MS is an emerging technology in this field[2]. For the analysis of peptides, CE/MS is an attractive complementary method to LC/MS, due to the efficient analysis of hydrophilic low molecular weight peptides[3,4]. Further, CE/MS has the additional advantage of having low sample volume requirements (1–20 nL) compared to LC/MS. In this context, this Application Note demonstrates a CE/MS method for the primary characterization of mAbs. Intact, heavy, and light chain fragments of mAbs were analyzed with the Agilent CE/MS solution for two of the mAb samples. The results of this work are contrasted to a recent publication that shows the analysis of the same samples by LC/MS[5].

Experimental

Samples

Monoclonal antibodies (IgG1 and IgG2) were proprietary therapeutic molecules.

Instrumentation

CE

Agilent 7100 CE System

MS

Agilent 6530 (option 200) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF)

Interface

Orthogonal triple tube sheath liquid interface (G1607B)

Software

MassHunter Acquisition Software (B.06) for data acquisition and BioConfirm software for data analysis. The raw MS spectra were subjected to pMod algorithms to generate the zero charge state spectra.

The CE/MS analysis was performed using the 7100 CE System with a CE/MS capillary cassette (G1603A) coupled to the 6530 Accurate-Mass Q-TOF equipped with electrospray source and orthogonal coaxial sheath liquid interface (G1607B). The sheath liquid was delivered by an Agilent 1200 series isocratic pump equipped with a 1:100 flow splitter. Table 1 shows the CE/MS parameters.

Table 1. Instrumental conditions.

Capillary electrophoresis (CE)	
CE	Agilent 7100 CE
Sample	mAbs (3 µg/µL)
Injection	10 seconds and 20 seconds at 50 mbar
Capillary	PVA, total length 70 cm, 50 µm id
Buffer	50 mM acetic acid
Voltage	30 kV
Internal pressure	20 mbar
Temperature	20 °C
Mass spectrometry (MS)	
MS	Agilent 6530 Accurate-Mass Q-TOF LC/MS
Ionization mode	ESI
Acquisition mode	MS (mass range 1,000–6,000 *m/z*)
Sheath liquid	0.5 % acetic acid in 50 % methanol, 5 µL/min
Drying gas flow	5 L/min
Nebulizer	10 psi
Drying gas temperature	250 °C
Fragmentor	350 V
Vcap	3,500 V

Results and Discussion

Intact mAb analysis

Figure 1 shows the CE/MS trace for the mAbs at intact levels. The mAbs were detected between 12 and 18 minutes as a broad peak (Figure 1A and 1B). The average mass spectrum of both intact mAbs are shown in Figures 1C and 1D. The charge state envelope spread between 2,400 *m/z*–5,100 *m/z*, higher in *m/z* than a typical LC/MS analysis using 0.1 % formic acid[5]. The mass spectrum was deconvoluted using the peak modeling (pMod) deconvolution algorithm in Agilent MassHunter BioConfirm Software, and the deconvoluted spectra are shown in Figures 1E and 1F. The deconvoluted spectra for both mAbs showed four major mass peaks corresponding to different glycoform species, which is in good agreement with LC/MS measurements[5].

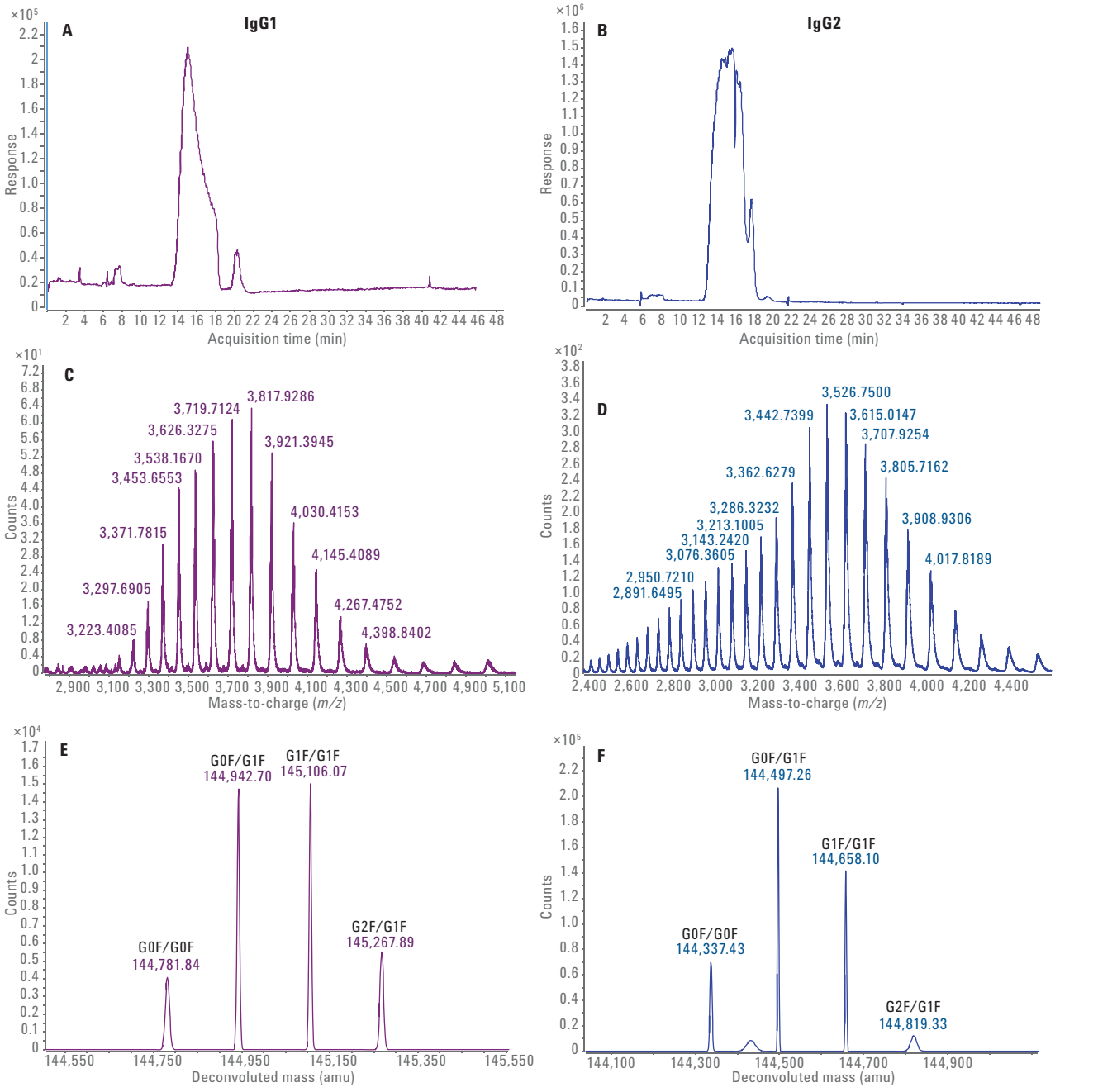

Figure 1. CE/MS of intact mAbs. A and B) total ion electropherograms of IgG1 and IgG2, C and D) charge envelope of IgG1 and IgG2, E and F) deconvoluted masses of IgG1 and IgG2.

Reduced mAb analysis

To study the mAb fragments, reduction of disulfides (with DTT) was performed to produce light and heavy chains of mAbs[5]. The total ion electropherogram and the deconvoluted spectra of the light chain (LC) and heavy chain (HC) of both mAbs are shown in Figures 2A–2F respectively. The CE/MS method showed reasonably good separation between LC and HC. The light chains of both IgG1 and IgG2 showed a single mass peak at 22,930.44 Da and 22,929.19 Da respectively (Figures 2C and 2D). LC/MS analysis of the same samples showed various satellite peaks near the light chain signal, corresponding to salt adducts[5]. Inspection of the heavy chain CE/MS deconvoluted spectrum shows multiple peaks assigned to glycan modifications (Figures 2E and 2F), which are identical to the glycoforms observed in the intact level, further validating the glycan assignment at the intact mass level.

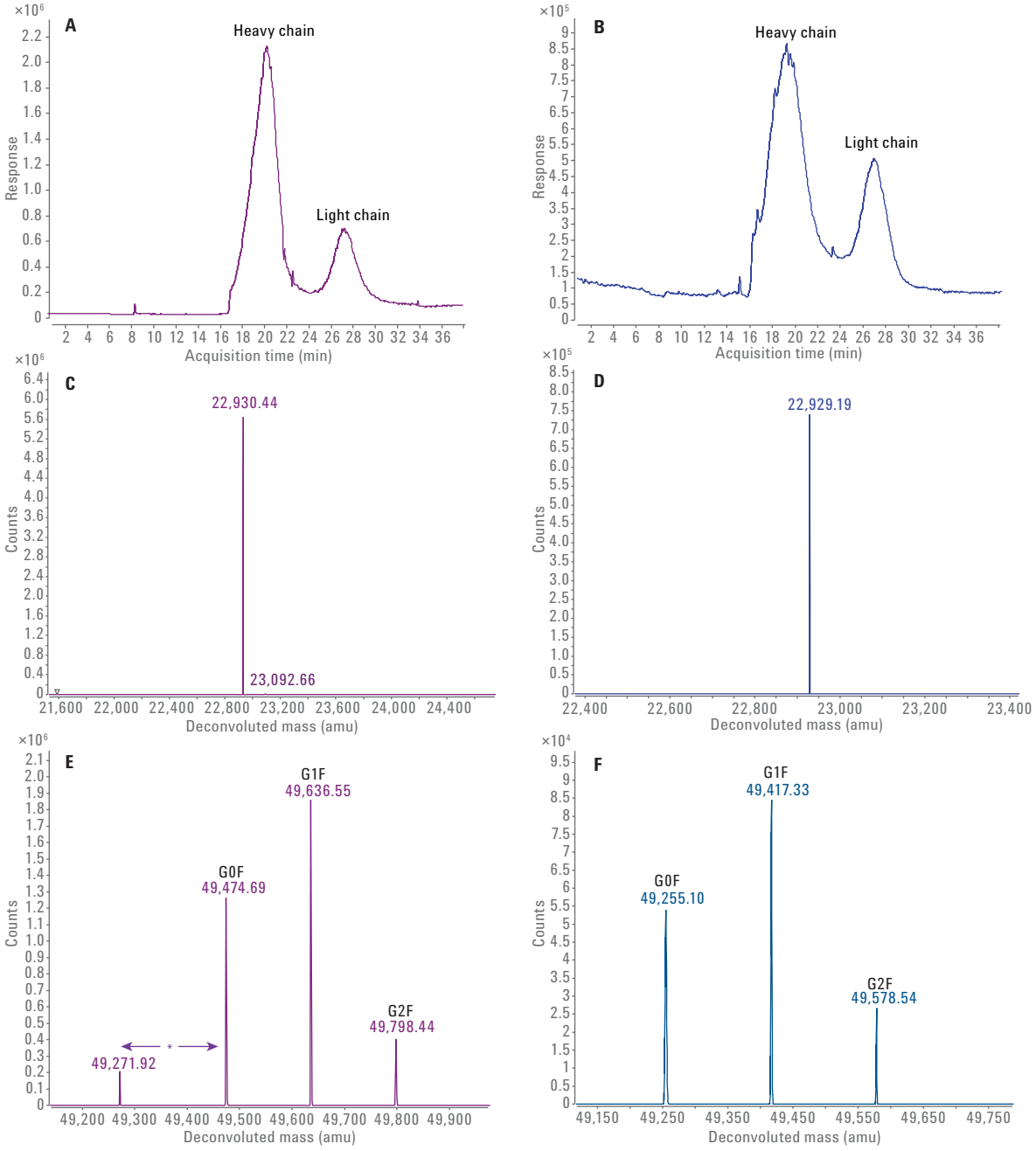

Figure 2. CE/MS of reduced mAbs. A and B) total ion electropherograms of reduced IgG1 and IgG2, C and D) deconvoluted masses of light chain of IgG1 and IgG2, E and F) deconvoluted masses of heavy chain of IgG1 and IgG2. *n*-acetyl glucosamine.

The CE/MS deconvoluted spectra of IgG1 heavy chain showed a peak with a mass difference of 203 Da, corresponding to *n*-acetyl glucosamine modification (*), which was absent in the LC/MS run. The results obtained are in good agreement with LC/MS measurements[5].

Conclusion

The characterization of mAbs using an Agilent 7100 CE System coupled to an Agilent 6530 Accurate-Mass Q-TOF has been showcased. The usefulness of CE/MS for the analysis of intact antibodies and its fragments was demonstrated by using two mAbs in the present study. This CE/MS method can be used as a complement to LC/MS for the routine analysis of mAbs.

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