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Intact mass analysis of monoclonal antibodies by capillary electrophoresis—Mass spectrometry

Mei Han*, Brooke M. Rock, Josh T. Pearson, Dan A. Rock

Pharmacokinetics and Drug Metabolism, Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA, USA

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ABSTRACT

Characterization of monoclonal antibody (mAb) therapeutics by intact mass analysis provides important information on sequence integrity and post-translational modifications. In order to obtain domain specific information, monoclonal antibodies are reduced to heavy and light chain components or enzymatically digested into smaller portions or peptides. Liquid chromatography (LC) is widely used for separation of the antibody fragments in line with mass spectrometry (MS) for characterization. Capillary electrophoresis (CE) is an analytical technique with high separation efficiency, high sensitivity, and minimal inter-run sample carryover. Combining the resolving power of CE with electrospray ionization (ESI) MS has great potential in regards to accurate mass characterization of protein therapeutics and has been a long sought-after approach. However, the intrinsic technical difficulty in coupling CE to MS has hindered the broad application of CE-MS across the biopharmaceutical industry. Recently, a CE-MS interface has been developed [1] and commercialized. Herein, we report implementation of this technology for coupling CE to an Agilent time-of-flight (TOF) mass spectrometer. CE-MS provides an attractive complement to LC–MS for separation and intact mass determination of mAbs and antibody-based therapeutics.

1. Introduction

Protein therapeutics and monoclonal antibodies (mAbs) are the fastest growing therapeutic class of drug candidates and products developed by biopharmaceutical companies around the world [2–4]. Due to the complexity of the manufacturing process of mAbs, modest modifications in expression or purification conditions can result in production of heterogeneous mAb preparations consisting of individual species having altered primary sequences and varying posttranslational modifications, during cell culture expression, purification, formulation and storage [5,6]. Since heterogeneity can impact the overall stability and biological activity of mAbs, a comprehensive characterization of the preparation is required [7]. Among the extensive analytical techniques, mass spectrometry has played a key role for antibody characterization with its capability of high resolution and mass accuracy measurements. Protein intact mass analysis is an important component in the characterization of recombinant mAb therapeutics [5,7–10]. Direct mass measurement allows for the assignment of chemical modifications contributing to the product heterogeneity. Measuring whole and partial molecular species within a mAb preparation to characterize protein amino acid sequence, identify domain specific modifications, profile glycoforms and evaluate possible changes under in vivo conditions are all effective ways to identify protein features that may be susceptible to modification and that might be attributes critical to stability, efficacy and safety profiles [11].

In order to effectively characterize protein therapeutics, a sample introduction system is necessary for mass spectrometry analysis. To date, this has been largely carried out through liquid chromatography (LC) separation or direct-infusion [9,10]. The wide availability of LC columns, well-developed analytical methods, and system robustness have made LC the standard introduction system for the characterization of protein therapeutics by MS. The commonly used chromatography approaches separate proteins by their differences in hydrophobicity (reversed phase, RP, hydrophobic interaction chromatography, HIC), charge (ion exchange, IEX), or size (size exclusive chromatography, SEC) [9,10,12,13]. There are

Abbreviations: CE, capillary electrophoresis; CE-MS, capillary electrophoresis-mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; IgG, immunoglobulin G; mAb, monoclonal antibody; TOF, time-of-flight; BGE, background electrolyte; BPE, mass spectrometry base peak electropherogram; LPA, linear polyacrylamide; PTMs, post-translational modifications; Fab, antigen-binding fragment; Fc, crystallizable fragment.

* Corresponding author.
E-mail address: hanm@amgen.com (M. Han).

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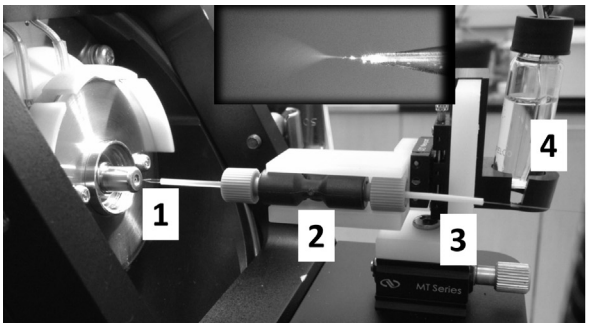

1. Spray emitter
2. Interface tee
3. Separation capillary
4. Sheath liquid vial

Fig. 1. CE-MS interface installed on an Agilent TOF instrument. The outlet of the separation capillary was placed at the spray emitter tip through the interface tee. The mass spectrometer capillary voltage was set to be zero volts. The ESI voltage was provided by an external power supply (not shown), which generated an electrical field from the sheath liquid vial to the mass spectrometer. As a result, a steady electroosmotic flow was formed on the inner surface of the borosilicate glass spray emitter, providing nanoflow of sheath liquid which entrains the analytes into the mass spectrometer. The inset shows the spray plume.

inherent challenges in each of these chromatography-based techniques [9]. For example, in a typical setting of reversed phase LC–MS separation of reduced monoclonal antibody, the column temperature has to be heated (70 °C) in order to achieve baseline separation between heavy and light chains. The elevated temperature can introduce artificial degradation of these partial molecules [12]. In addition, protein retaining on the LC columns often leads to peak tailing and sample carry-over. Moreso, developing chromatography with consistent peak shapes and baseline separation of the heavy and light chains in an antibody can be time consuming with optimal conditions varying from antibody to antibody [13–15]. The impact of protein structure on pharmacokinetics has increased the need for higher protein separation efficiency combined with intact mass spectral analysis in order to characterize pharmacokinetics for biologics [9,11].

Capillary electrophoresis (CE) represents an exciting complementary separation technique to LC for proteins and antibodies. CE offers selectivity, high resolving power, and fast separation speed [16,17]. CE is a widely used separation technique for the characterization of biotherapeutics for size, charge and glycan analysis [18–21]. Numerous robust assays have been established to analyze monoclonal antibodies, such as CE-SDS in the evaluation of product-related impurity, capillary isoelectric focusing (cIEF) for charge variants analysis, and CZE for released glycan analysis with either UV or Laser Induced Fluorescence (LIF) detection [18–21]. With the success of these optical detection based methods established, it is appealing that coupling CE separation to online electrospray ionization mass spectrometers as a detector to gain additional sensitivity and accurate mass identification [22–27]. However, due to the technical difficulties in coupling CE to MS, CE-MS has yet to be routinely paired with LC–MS as an alternative or complementary separation technique for protein characterization. A significant challenge for introducing samples from CE into the mass spectrometer involves the need of electrical contact between the electrophoretic separations from CE to the electrospray ion source. Currently, there are two major types of CE-MS interface—sheath liquid interface and sheathless interface [22,23,28–30]. Sheath liquid interface coaxially adds sheath liquid at the end of the CE separation capillary to provide electrical contact therefore assisting the spray. Sheath liquid can also be modified for improving electrospray efficiency [1,22,23]. However, the sensitivity is scarified due to the high dilution rate (10 to 100× fold) of analytes by sheath liquid. In addition, peak broadening might occur due to diffusion of analytes [29]. The sheathless interface overcomes the sample dilution issue by eliminating the sheath liquid. The CE separation capillary tip was etched and inserted into the stainless steel electrospray needle filled with conductive liquid [29,30] to establish the electrical contact improving the overall sensitivity. However, sheathless interfaces can lead to instability in the electrospray and needs to be carefully monitored during operation. While this system has definite advantages, the choice of CE instrument is limited [1,29]. Recently, a nanospray sheath flow interface was designed by Dr. Dovichi's lab [1] which utilize electroosmotic flow (EOF) on the inner surface (electrokinetic flow) of borosilicate glass emitter to drive the sheath liquid, has greatly enhanced the sensitivity and spray performance [1].

Current CE-MS interface technology has broadened the scope of the technology in proteomics, metabolomics, and protein characterization particular of complex proteins such as mAbs peptides, protein digest, protein complexes as well as some intact protein analysis [1,29–35]. Here, we describe coupling CE to a time-of-flight (TOF) mass spectrometer using the electrokinetic flow driven nanospray sheath liquid interface described above [1,32]. A mix of commonly used protein standards was utilized to identify optimal source conditions for introducing protein samples into the TOF instrument after separation by CE. With optimal source conditions defined, we tested the utility of this novel CE-TOF platform to measure the intact mass for some representative fully human mAbs and their fragments under native and reducing conditions. Under reducing conditions we were able to demonstrate baseline separation of antibody heavy and light chains with accurate mass determination by TOF MS without the need for elevated temperature during sample separation. CE-MS provides an attractive complement to LC–MS for separation and intact mass determination of mAbs and antibody-based therapeutics. The setup and use of CE-MS for protein characterization is discussed in detail.

2. Materials & methods

2.1. Materials and chemicals

Monoclonal antibodies were manufactured at Amgen (Thousand Oaks, CA). Myoglobin Ribonuclease A, Cytochrome C, Insulin and all other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). The 1× PBS buffer (phosphate buffered saline) was purchase from Life Technologies (Grand Island, NY). PNGase F enzyme was obtained from New England BioLabs Inc. (Ipswich, MA). IdeS enzyme was purchased from Genovis Inc. (Cambridge, MA). Bio-Rad Bio-Spin P-6 column was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). LPA-coated capillaries and borosilicate glass spray emitters were purchased from CMP Scientific, Corp. (Brooklyn, NY). Ultra-pure water was generated in-house from a Milli-Q Plus system from Millipore.