Journal of Chromatography A, 1359 (2014) 303–308

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Capillary zone electrophoresis-electrospray ionization-tandem mass spectrometry for quantitative parallel reaction monitoring of peptide abundance and single-shot proteomic analysis of a human cell line☆

Liangliang Sun, Guijie Zhu, Si Mou, Yimeng Zhao, Matthew M. Champion, Norman J. Dovichi*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

ARTICLE INFO

Article history:
Received 9 April 2014
Received in revised form 25 June 2014
Accepted 10 July 2014
Available online 17 July 2014

Keywords:
CZE-ESI-MS/MS
CZE-PRM
Ultrasensitive electrokinetically pumped sheath flow nanospray interface
Shotgun proteomics
Human cell lysate digest

ABSTRACT

We coupled capillary zone electrophoresis (CZE) with an ultrasensitive electrokinetically pumped nanospray ionization source for tandem mass spectrometry (MS/MS) analysis of complex proteomes. We first used the system for the parallel reaction monitoring (PRM) analysis of angiotensin II spiked in 0.45 mg/mL of bovine serum albumin (BSA) digest. A calibration curve was generated between the loading amount of angiotensin II and intensity of angiotensin II fragment ions. CZE-PRM generated a linear calibration curve across over 4.5 orders of magnitude dynamic range corresponding to angiotensin II loading amount from 2 amole to 150 fmole. The relative standard deviations (RSDs) of migration time were <4% and the RSDs of fragment ion intensity were ~20% or less except 150 fmole angiotensin II loading amount data (~36% RSD). We further applied the system for the first bottom up proteomic analysis of a human cell line using CZE-MS/MS. We generated 283 protein identifications from a 1 h long, single-shot CZE MS/MS analysis of the MCF7 breast cancer cell line digest, corresponding to ~80 ng loading amount. The MCF7 digest was fractionated using a C18 solid phase extraction column; single-shot analysis of a single fraction resulted in 468 protein identifications, which is by far the largest number of protein identifications reported for a mammalian proteomic sample using CZE.

1. Introduction

Capillary electrophoresis (CE)-electrospray ionization (ESI)-mass spectrometry (MS) has been used to characterize a wide range of analytes, including intact proteins, peptides, metabolites, etc. [1–4]. CE-ESI-MS applications have benefited from recent improvements in the electrospray interface [5]. As a result of these improvements, CE-MS has attracted renewed interest as a tool for proteomics research [6–8].

There are several important recent developments in CE-MS interface designs. Moini reported a sheathless capillary electrophoresis–electrospray interface in 2007 [9], which employed a porous capillary tip as the nanospray emitter. The sheathless interface system has been used for analysis of peptides [10,11], Arg-C-digested histones, a *Pyrococcus furiosus* tryptic digest [12–14], and intact proteins [15,16].

A second CE-nanospray interface was reported by our group in 2010 [17]. This sheath-flow interface employs a glass emitter with a ~5-μm orifice. Electro-osmosis at the glass surface drives the sheath fluid at very low rates. The interface has several advantages, including minimal sample dilution due to the very low sheath flow rate, elimination of mechanical pumps and nebulizing gas, use of a wide range of separation buffers, and stable operation in the nanospray regime. We have applied the electrokinetically pumped sheath flow nanospray interface CE-MS/MS system for shot-gun proteomic analysis of the secretome of *Mycobacterium marinum* [18], a fraction of yeast lysate [19], the *Escherichia coli* proteome [20–22], picogram amounts of RAW 264.7 cell lysate [23–25], and the PC12 cell lysate [26]. In addition, the system was also applied for top-down intact protein characterization [27], quantitative multiple reaction monitoring (MRM) of peptide abundance [28], and phosphopeptides characterization [29].

Very recently, we reported a simple modification to our interface that resulted in ultrasensitive performance. We etched a few millimeters of the outside of the separation capillary tip with hydrofluoric acid to reduce its outer diameter from ~150 μm to ~60 μm. This step allows the capillary tip to be

☆ Invited paper for the Honor Issue of Professor Peichang Lu's 90th birthday.

* Corresponding author. Tel.: +1 574 631 2778.
E-mail address: ndovichi@nd.edu (N.J. Dovichi).

http://dx.doi.org/10.1016/j.chroma.2014.07.024
0021-9673/

placed within 200 μm of the emitter orifice, which results in a significant improvement in the system's sensitivity [30]. By employing 10 μm i.d. separation capillary, a Q-Exactive mass spectrometer, and the improved CE-MS interface, we obtained 1 zmole (1 zmol = 10^{-21} mol = 600 molecule) peptide detection limit ($S/N = 3$). Over 100 proteins were identified based on tandem mass spectra from 16 pg of *E. coli* digest and 154 peptides from 60 proteins were identified from 400 fg sample loading.

Besides the interfaces mentioned above, the flow-through microvial interface developed by Chen group is also widely used for metabolomic [31], glycan [32], and intact protein [33] analysis. In addition, Tang's group recently developed a novel sheathless CE-MS interface combining a large i.d. separation capillary and a detachable small i.d. porous ESI emitter [34]. This design combines a large sample loading volume with stable nanoESI operation, and the design has produced a 10 pM limit of quantification for standard peptides spiked in a BSA tryptic digest background.

CE-MS/MS has a brief history for analysis of complex protein digests. Yates' group developed a solid-phase microextraction technique to prefractionate a yeast ribosome digest followed by CE-MS/MS analysis in 1999; 66 proteins were identified using an ion trap mass spectrometer from the eight fractions [35]. Recently, Yates' group reported an improved on-line microextraction fractionation, transient isotachophoresis capillary electrophoresis–MS/MS system. They employed an etched porous capillary as the ESI sprayer to analyze a tryptic digest of *Pyrococcus furiosus*; 548 protein IDs were obtained in duplicate separations of eight factions [13]. Lindner's group employed a capillary zone electrophoresis (CZE)-MS/MS system with the porous capillary tip based sheathless interface for analysis of a rat testis linker histone protein sample digested by endoproteinase Arg-C, and eight non-histone H1 proteins were identified [12].

Our group used the electrokinetically pumped sheath flow interface for the capillary zone electrophoresis analysis of the secretome of *M. marinum* in 2012; 140 protein groups were identified [18]. We improved the peptide separation by using linear polyacrylamide coated capillary and stacking injection; ~300 protein groups were identified from 100 ng of *E. coli* digests by single shot analysis in less than 1 h [20]; the number of protein IDs was increased to 871 by analyzing seven *E. coli* digest fractions from offline C18-SPE fractionation [21]. We also employed a capillary isoelectric focusing MS/MS system with the electrokinetically pumped sheath flow interface for eight-plex iTRAQ based quantitative proteomic analysis of differentiating PC12 cells; 835 protein groups were identified [26]. To our knowledge, there are no publications employing CZE-MS/MS for analysis of a human cell line.

For target proteomics research, multiple/selected reaction monitoring (MRM/SRM) is typically employed with triple-quadrupole (QqQ) mass spectrometer [36,37]. Briefly, the parent ion of a targeted peptide is isolated in the first quadrupole (Q1) and then fragmented in the second quadrupole (Q2). One or several fragment ions from the targeted peptide are further isolated by the third quadrupole (Q3) for detection [38,39]. Recently, Coon's group introduced a new target proteomics technique, named parallel reaction monitoring (PRM), which was performed with a benchtop quadrupole-Orbitrap mass spectrometer [40]. For PRM, a target peptide was selected in the quadrupole and then fragmented in the collisional cell. The resulting fragment ions were analyzed in the Orbitrap to generate one full, high-resolution MS/MS spectrum. Because m/z ratios of fragment ions are not required during the method development step, the process is much easier than SRM/MRM. In addition, PRM has much better tolerance to the background matrix than SRM/MRM due to the high resolution of the Orbitrap analyzer [40].

In this work, we present the first example of CZE-PRM. We employ our improved electrokinetically pumped sheath flow nanospray interface [30] for peptide analysis. A standard peptide, angiotensin II, was spiked in a 0.45 mg/mL bovine serum albumin digest to evaluate the CZE-PRM system performance. We observed over four and a half orders of magnitude linear dynamic range for angiotensin II corresponding to loading amounts from 2 to 150,000 amole. We also presented the first example of CZE-MS/MS for bottom-up analysis of a human cell line; nearly 300 proteins were identified from MCF7 whole cell lysate digest in a 1-h single-shot CZE-MS/MS analysis with ~80 ng loading amount.

2. Experimental

2.1. Materials and reagents

Bovine pancreas TPCK-treated trypsin, bovine serum albumin (BSA), urea, ammonium bicarbonate (NH_4HCO_3), dithiothreitol (DTT), iodoacetamide (IAA), and angiotensin II (human, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) were purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile (ACN), formic acid (FA), and hydrofluoric acid (HF) were purchased from Fisher Scientific (Pittsburgh, PA). Methanol and water were purchased from Honeywell Burdick & Jackson (Wicklow, Ireland). Fused silica capillary (10 and 20 μm i.d./150 μm o.d.) and linear polyacrylamide (LPA) coated capillary (50 μm i.d./150 μm o.d.) were purchased from Polymicro Technologies (Phoenix, AZ).

Eagle's minimal essential medium (EMEM), fetal bovine serum (FBS), GlutaMAX™ (100×), insulin, and Antibiotic-Antimycotic (Anti-Anti, 100×) were purchased from Life Technologies Corporation (Grand Island, NY). Mammalian Cell-PE LB™ buffer for cell lysis was purchased from G-Biosciences (St. Louis, MO). Complete, mini protease inhibitor cocktail (provided in EASYpacks) was purchased from Roche (Indianapolis, IN).

2.2. Sample preparation

Bovine serum albumin (BSA, 0.5 mg/mL) in 100 mM NH_4HCO_3 (pH 8.0) containing 8 M urea was denatured at 37 °C for 30 min, followed by standard reduction and alkylation with DTT and IAA. After dilution with 100 mM NH_4HCO_3 (pH 8.0) to reduce the urea concentration below 2 M, protein digestion was performed for 12 h at 37 °C with trypsin at a trypsin/protein ratio of 1/30 (w/w). After acidification, the protein digest was desalted with a C18-SepPak column (Waters, Milford, MA), followed by lyophilization with a vacuum concentrator (Thermo Fisher Scientific, Marietta, OH). The dried sample was dissolved in 0.05% (v/v) FA to produce a 0.5 mg/mL solution and stored at −20 °C before use.

Angiotensin II solution was spiked into the BSA digest to generate five samples in 0.05% (v/v) FA containing 0.45 mg/mL BSA digest and different concentrations of angiotensin II (10 nM, 100 nM, 1 μM, 10 μM and 100 μM). Each sample was analyzed by CZE-PRM in triplicate.

The procedures for MCF7 cell culture, cell lysis, protein acetone precipitation, denaturation, reduction and alkylation were described before [41]. Briefly, after cell culture, MCF7 cells were lysed by sonication, followed by BCA protein concentration measurement, and acetone precipitation. Then, a 690 μg protein aliquot was dissolved in 8 M urea and 100 mM NH_4HCO_3 (pH ~8.0), denatured at 37 °C for 1 h, and reduced in ~40 mM DTT at 37 °C for 1.5 h, followed by alkylation with 100 mM IAA at room temperature for 30 min. After dilution with 100 mM NH_4HCO_3 (pH ~8.0) to reduce the urea concentration below 2 M, the proteins were digested by trypsin at a trypsin/protein ratio of 1/30 (w/w) overnight at 37 °C. The protein digest was acidified with FA (1% final concentration), and desalted with C18-SepPak column (Waters, Milford, MA), followed by lyophilization. The peptide mixture was dissolved in