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Optimization of mass spectrometric parameters improve the identification performance of capillary zone electrophoresis for single-shot bottom-up proteomics analysis

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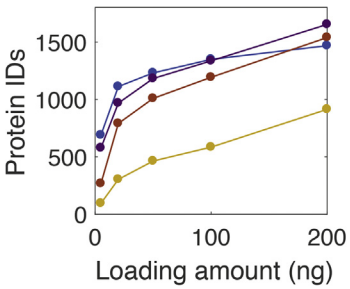
HIGHLIGHTS

- A programmable autogain control method produced best results for CZE analysis of *Xenopus* tryptic digest.
- 1,653 protein groups identified from 200 ng *Xenopus laevis* tryptic digest.
- First report where the protein IDs for CZE surpasses that of the UPLC for 200 ng samples.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of MS1 injection time, MS2 injection time, dynamic exclusion time, intensity threshold, and isolation width were investigated on the numbers of peptide and protein identifications for single-shot bottom-up proteomics analysis using CZE-MS/MS analysis of a *Xenopus laevis* tryptic digest. An electrokinetically pumped nanospray interface was used to couple a linear-polyacrylamide coated capillary to a Q Exactive HF mass spectrometer. A sensitive method that used a 1.4 Th isolation width, 60,000 MS2 resolution, 110 ms MS2 injection time, and a top 7 fragmentation produced the largest number of identifications when the CZE loading amount was less than 100 ng. A programmable autogain control method (pAGC) that used a 1.4 Th isolation width, 15,000 MS2 resolution, 110 ms MS2 injection time, and top 10 fragmentation produced the largest number of identifications for CZE loading amounts greater than 100 ng; 7218 unique peptides and 1653 protein groups were identified from 200 ng by using the pAGC method. The effect of mass spectrometer conditions on the performance of UPLC-MS/MS was also investigated. A fast method that used a 1.4 Th isolation width, 30,000 MS2 resolution, 45 ms MS2 injection time, and top 12 fragmentation produced the largest number of identifications for 200 ng UPLC loading amount (6025 unique peptides and 1501 protein groups). This is the first report where the identification number for CZE surpasses that of the UPLC at the 200 ng loading level. However, more peptides (11476) and protein groups (2378) were identified by using UPLC-MS/MS when the sample loading amount was increased to 2 μg with the fast method. To exploit the fast scan speed of the Q-Exactive HF mass spectrometer, higher sample loading amounts are required for single-shot bottom-up proteomics analysis using CZE-MS/MS.

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1. Introduction

Capillary zone electrophoresis-tandem mass spectrometry (CZE-MS/MS) is attracting increasing attention for proteomics analysis due to its high sensitivity [1–3], fast separation speed [4], small volume sample consumption, orthogonality to reversed-phase liquid chromatography (RPLC), and performance with mass-limited samples [5–7]. A number of recent papers have focused on improvements in sample preparation [8], preconcentration [9–11], and fractionation for CZE-based bottom-up proteomics [6,12].

Mass spectrometric parameters have been investigated to improve the identification rates for single-shot bottom up proteomics using RPLC-MS/MS [13–21]. To the best of our knowledge, there has been no published report that studied the effects of the mass spectrometric parameters on the identification performance of CZE-MS/MS method for bottom up proteomics analysis. In this work, we investigated the effects of mass spectrometric parameters on peptides and protein identification rates for single-shot bottom up proteomics analysis by using CZE-MS/MS and compared those results with RPLC-MS/MS, each operating under their optimized conditions. The results demonstrate that the optimized mass spectrometric parameters are not only related to the sample loading amount but also to the separation method.

2. Experimental section

2.1. Reagents and chemicals

Formic acid (FA), acetic acid (HOAc), bovine pancreas TPCK-treated trypsin, dithiothreitol (DTT), and iodoacetamide (IAA) were purchased from Sigma-Aldrich (St. Louis, USA). Methanol was purchased from Honeywell Burdick & Jackson (Wicklow, Ireland). Uncoated fused silica capillary (50 μm i.d. × 350 μm o.d.) was purchased from Polymicro Technologies (Phoenix, AZ). Water was deionized by a Nano Pure system from Thermo Scientific (Marietta, OH).

2.2. Preparation of Xenopus laevis sample

The method used for the preparation of the *Xenopus laevis* tryptic digest is described in Supporting Information.

2.3. Preparation of linear polyacrylamide coated capillary

The linear polyacrylamide (LPA) coated capillary was prepared by using a surface-confined aqueous reversible addition-fragmentation chain transfer (SCARAFT) polymerization method [22].

2.4. CZE-ESI-MS/MS analysis

A PrinCE Next 840 Series autosampler (from PrinCE Technologies) was used for sample injection and CZE separation. The sample was injected by pressure. Separation voltage was applied at the injection end of the capillary with the autosampler. An electrokinetically pumped nanospray interface was used to couple the CZE separation capillary to a Q Exactive HF mass spectrometer (Thermo Scientific). The electrospray emitter was made from a borosilicate glass capillary (1.0 mm o.d. × 0.75 mm i.d., 10 cm long) pulled with a Sutter instrument P-1000 flaming/brown micropipette puller; the size of the emitter opening was 15–20 μm. The electrospray sheath electrolyte was 10% (v/v) methanol with 0.5%FA. The background electrolyte was 1 M HOAc in water. The 50 μm i.d. × 350 μm o.d. × 99 cm LPA coated capillary was used as the separation capillary. The *Xenopus laevis* proteins digest dissolved in 30 mM NH_4HCO_3 was used as the standard sample. 1.6 kV was applied to the sheath flow reservoir for electrospray. The mass spectrometer's operating parameters are described below.

2.5. UPLC-ESI-MS/MS analysis

An ACQUITY UPLC M-Class system (Waters, Milford, MA, USA) with an ACQUITY UPLC M-Class Peptide BEH C18 column (Waters, 100 μm × 100 mm, 1.7 μm, 300 Å) was coupled to a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) for peptide separation and identification. Mobile phase A (0.1%FA in water) and mobile phase B (0.1% FA in ACN) were used for gradient separation. Peptides were automatically loaded onto a commercial C18 reversed phase column and flushed with 2% mobile phase B for 10 min at a flow rate of 0.9 μL/min, then followed by the gradient: 10–12 min, 2–8% B; 12–70 min, 5–30% B; 70–74 min, 20–80% B; 74–79 min, 80% B; 79–80 min, 80–2% B; 80–90 min, 2% B. The eluted peptides from the C18 column were pumped through a capillary tip for electrospray.

2.6. Mass spectrometer operating parameters

A Q Exactive HF mass spectrometer (Thermo Scientific) was used in this work. The mass spectrometer was programmed in data-dependent mode. The S-lens RF level was set at 60, and heated capillary at 300 °C. Full scan resolution was set to 60,000 at m/z 200. Full scan target was 3.00E + 06. Mass range was set to m/z 350–1800. Target value for fragment scans was set at 1.00E + 06. A fixed first mass of 100 was used. Normalized collision energy was set at 28. Other parameters are optimized in this work.

2.7. Database searching

Database searching of the raw files was performed in both Proteome Discoverer 1.4 (Thermo) with MASCOT 2.5 and Maxquant 1.5.8.3. The *Xenopus laevis* database (version 9.1) was downloaded from Xenbase (http://www.xenbase.org/, RRID:SCR_003280). Database searching for the reversed database was also performed to evaluate the false discovery rate. The database searching parameters included full tryptic digestion and allowed up to two missed cleavages, the precursor mass tolerance was set at 10 ppm, and fragment mass tolerance was 0.05 Da. Carbamidomethylation (C) was set as a fixed modification. Oxidation (M) and deamidated (NQ) were set as variable modifications. On the peptide level, peptide confidence value as high was used to filter the peptide identification, and the corresponding false discovery rate on peptide level was less than 1%. On the protein level, protein grouping was enabled.

3. Results and discussion

We investigated the effect of a number of mass spectrometer operating parameters on peptide and protein identifications in the CZE-MS/MS analysis of the *Xenopus* embryo proteome.

3.1. Effect of MS1 injection time

Fig. 1 presents the total ion electropherogram for the injection of 100 ng of *Xenopus laevis* embryo tryptic digest. The first components migrate at roughly 20 min and the last components migrate at 100 min. The figure also presents the MS1 injection time for the separation of this sample. The fill time minimizes at ~2 ms for the portion of the electropherogram with the highest base-peak intensity, and reaches a maximum value of ~14 ms for the lowest

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