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A novel approach for quantitative peptides analysis by selected electron transfer reaction monitoring

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ABSTRACT

Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) with selective reaction monitoring (SRM) is a selective and sensitive method for quantitation of peptides. SRM is achieved via MS/MS utilizing collision-induced dissociation (CID) while monitoring unique precursor-product ion transitions. Low-energy CID tandem mass spectrometry has been, by far, the most common method used to dissociate peptide ions for sequence analysis. However, collisional scattering of product ions in CID results in decreased intensity of the primary product ion. The lower intensity of the targeted product ion can lead to a reduction in the sensitivity of a quantitative method that uses SRM. Electron transfer dissociation (ETD) is a fragmentation method that is complementary to CID. During the ETD reaction for doubly protonated peptides ($[M+2H]^{2+}$), there is a significant shift toward nondissociative electron transfer (ET) product species ($[M+2H]^{+\bullet}$). We utilized that particular defect in ETD to develop a new quantitative method for monitoring the transition of unique precursors ($[M+2H]^{2+}$) to charge-reduced ions ($[M+2H]^{+\bullet}$). We refer to this method as selective electron transfer reaction monitoring (SETRM). In ESI-MS, trypsin-digested peptides tend to generate doubly protonated peptide precursors. We found that SETRM was more suitable than SRM for these doubly charged tryptic peptides with nano-LC–MS/MS. The quantitative capabilities of SETRM provide a more sensitive way of performing quantitative experiments using the same instrument, thereby improving the application of electron transfer dissociation in proteomics.

1. Introduction

The application of peptides as drugs in therapeutic treatment, or as biomarkers in clinical diagnosis is becoming more widespread. The establishment of a sensitive and accurate analytical methodology is indispensable for quantitative assessment of peptides. Mass spectrometric-based quantitative analysis has a high specificity for detecting peptide components in complex biological matrices such as plasma, urine, and tissue [1–5].

In the most common application of mass spectrometric-based quantitative analysis, specific peptides are measured using selected reaction monitoring (SRM) [4–6]. That technique monitors the product ion resulting from collision-induced dissociation (CID) of selected precursor ions when the sample is introduced into the MS/MS instrument. The sensitivity gains result from the greater signal-to-noise (S/N) ratio that is the characteristic of MS/MS. During CID processes, however, the fragmented product ions are dispersed, leading to a reduction in the number of ions reaching the detector and hence a reduction in the sensitivity of SRM-based measurement methods [7–9].

Electron transfer dissociation (ETD) is a recently introduced mass spectrometric technique that has been shown to complement CID. In ETD, multiple protonated peptide ion species react with negative ions, such as fluoranthene. The reaction induces fragmentation of the peptide backbone, causing cleavage of the N–Cα bond. This creates complementary c-type and z-type fragment ions [10–12]. ETD is advantageous over CID because it is less dependent on cleavage site sequences and because it preserves labile post-translational modifications (PTMs), such as phosphorylation and glycosylation [13–17].

Although the dissociation efficiency of ETD is not dependent on peptide length, amino acid composition, or post-translational modifications, it is dependent on the charge state of the precursor ion [18]. During ETD, highly charged precursor ions (charge state $\geqq 3$) produce a series of c ions and complementary z ions, thereby allowing for almost complete sequence coverage. Fragmentation of doubly charged precursors is often limited to one or both ends of the peptide, and there is an apparent phenomenon toward electron

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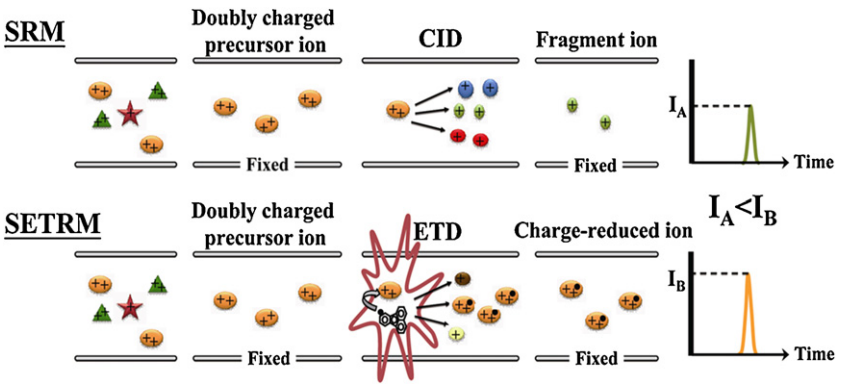

Fig. 1. SRM vs. SETRM in doubly-charged peptide quantitation.

transfer as a function of reducing precursor ion charge [19–22]. The electron transfer reaction leads into connection between c ions and complementary z ions with noncovalent intramolecular interactions. The precursor ion charge is reduced by this electron transfer reaction [23]. Most proteomic approaches, however, rely on tryptic peptides that produce primary doubly charged ions. Improving the dissociation efficiency of ETD for doubly charged precursor ions might be possible by elevating the temperature or by collisional activation [18,20,23,24].

To our knowledge, this is the first report of using electron transfer reaction in the quantitation of doubly charged peptides. In this study we evaluated the effectiveness of selective electron transfer reaction monitoring (SETRM) of charge-reduced precursor ions, tryptic peptides especially (Fig. 1).

2. Experimental

2.1. Chemicals and reagents

[Glu^1]-Fibrinopeptide B peptide, somatostatin, angiotensin I, ACTH 1–17, and ACTH 7–38 and MiniTip^{TM} C_{18} were purchased from Sigma–Aldrich (St. Louis, MO). Bovine serum albumin (BSA) was purchased from Pierce (Rockford, IL). BSA digested standard was purchased from Waters (Milford, MA). Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Formic acid (FA) and trifluoroacetic acid (TFA) were purchased from Fluka (Buchs, Switzerland). All samples were used without further purification.

2.2. In-solution digestion

BSA was diluted in 50 mM NH_4HCO_3 to reach a final concentration of 100 μg/mL. Disulfide bonds were reduced with 10 mM dithiothreitol for 1 h at 56 °C, and then alkylated with 50 mM iodoacetamide for 30 min at room temperature in the dark. Subsequently, alkylated proteins were digested with trypsin at a ratio of 1:20 (w/w, trypsin/protein), and incubated at 37 °C overnight. After digestion, the peptides were dried in a vacuum centrifuge, and then resuspended in 10 μL of 0.1% TFA for MiniTip^{TM} C_{18} desalting.

2.3. Liquid chromatography and mass spectrometry

Synthetic peptides were dissolved in 50% acetonitrile with 0.1% formic acid and infused into the mass spectrometer via an electrospray source. Anaylates for LC–MS/MS analyses were introduced into the mass spectrometer via high-performance liquid chromatography using an Agilent (Palo Alto, CA) 1200 series binary HPLC pump and an LC packings FAMOS^{TM} well-plate microautosampler. For each analysis, sample was loaded into a 2 cm × 75 μm i.d. trap column. The trap column was connected to a 11 cm × 75 μm i.d. analytical column and the columns were rigidly packed in-house with 5 μm C_{18} reversed-phase packing material. Mobile phase A consisted of 0.1% formic acid and mobile phase B consisted of 0.1% formic acid in 100% acetonitrile. The flow rate was 250 nL/min with linear gradients of: (1) 5–40% B for 27 min; (2) 40–85% B for 3 min; (3) isocratic at 85% B for 10 min.

All mass spectrometric analyses were performed on a ThermoFisher Scientific LTQ XL (San Jose, CA) linear ion trap mass spectrometer equipped with a chemical ionization source for the generation of radical anions (fluoranthene) for ETD reactions. For both CID and ETD analyses, precursor ion width was set at 2 Da and the automatic gain control (AGC) of the precursor cations for MS^n scan was set at 1×10^5. For the CID experiment, the *q*-value was equal to 0.25 and the normalized collision energy ranged from 0% to 35%. For the ETD experiment, the reaction time ranged from 50 ms to 300 ms and the AGC target for fluoranthene anions ranged from 1×10^5 to 3×10^5.

2.4. Quantitative analysis

For [Glu^1]-Fibrinopeptide B human peptide, the SRM transition was *m/z* 786.2 (doubly charged precursor ion) → 480.3 (y_4 ion) and the SETRM transition was *m/z* 786.2 (doubly charged precursor ion) → 1571.3 (charge-reduced ion). A standard curve was established using different amounts of [Glu^1]-Fibrinopeptide B human peptide (500 amol–1 pmol) and the area under the curve was calculated for all transitions using the vendor-supplied Xcalibur data system. Method validation was carried out using a set of standards (0.5–50 fmole) analyzed in triplicate. For the matrix effect test, a 25 μL aliquot of [Glu^1]-Fibrinopeptide B human peptide (1 fmol/μL) prepared in 50 mM NH_4HCO_3 was evaporated using a speed vacuum concentrator and then dissolved in 10 μL of 0.1% TFA for MiniTip^{TM} C_{18} desalting.

2.5. Desalting peptides with MiniTip^{TM} C_{18}

The 10 μL MiniTip^{TM} pipette tips contain a C_{18} spherical silica (50–60 μm, 200 Å pore size) sorbent bed bonded at the working end of the tip, were used for the desalting of the peptides. The tips were first wetted with 10 μL of 0.1% TFA in 70% ACN, then were equilibrated with 10 μL of 0.1% TFA. The peptides dissolved in 10 μL of 0.1% TFA, then were bound to MiniTip^{TM} by fully depressing the pipette plunger to a dead stop. Samples were aspirated and dispensed for 10 cycles. Then the tips were washed with 10 μL of 0.1% TFA twice. The peptides on the tips were eluted out using 10 μL of 0.1% TFA in 70% ACN. After desalting, the peptides were dried in a vacuum centrifuge, and then resuspended in 0.1% FA (25 μL for [Glu^1]-Fib B) before MS analysis.