International Journal of Mass Spectrometry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Protein derivatization and sequential ion/ion reactions to enhance sequence coverage produced by electron transfer dissociation mass spectrometry

Lissa C. Anderson [a], A. Michelle English [a], Wei-Han Wang [a], Dina L. Bai [a], Jeffrey Shabanowitz [a], Donald F. Hunt [a,b,*]

[a] University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904-4319, USA
[b] Department of Pathology, Health Sciences Center, University of Virginia, Charlottesville, VA 22908, USA

ARTICLE INFO

Article history:
Received 4 April 2014
Accepted 27 June 2014
Available online xxx

Keywords:
ETD
IIPT
Protein derivatization

ABSTRACT

Previously, we described implementation of a front-end ETD (electron transfer dissociation) source for an Orbitrap instrument [1]. This source facilitates multiple fills of the C-trap with product ions from ETD of intact proteins prior to mass analysis. The result is a dramatic enhancement of the observed ion current without the need for time consuming averaging of data from multiple mass measurements. Here we show that ion–ion proton transfer (IIPT) reactions can be used to simplify ETD spectra and to disperse fragment ions over the entire mass range in a controlled manner. We also show that protein derivatization can be employed to selectively enhance the sequence information observed at the N- and C-termini of a protein.

1. Introduction

Since the discovery and use of electron capture dissociation (ECD) by Zubarev, Kelleher and McLafferty on a Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer in 1998 [2], much progress has been made in the development of mass spectrometric methods for the analysis of intact proteins [3–6]. Electron transfer dissociation (ETD) was implemented on less expensive, quadrupole linear ion trap (QLT) instruments in 2004 [7] and proved to be ideally suited for the characterization of large peptides modified by phosphorylation [8] or O-GlcNAcylation [9]. ETD of intact proteins on a QLT instrument was first attempted in 2005 [10]. Proteins were converted to multiply charged positive ions by electrospray ionization and then allowed to react with fluoranthene radical anions. Electron transfer to the multiply charged proteins caused extensive fragmentation. Multiply charged fragment ions were then deprotonated by a second reaction with the carboxylate anion of benzoic acid and the resulting singly and double charged ions were used to read the amino acid sequence at both ends of the protein. With this information and the mass of the intact molecule it was possible to search protein databases for possible matches and detect the presence of posttranslational modifications or splice variants. A 2007 paper described the use of this technology to analyze intact proteins from the *E. coli*, 70S ribosomal protein complex [11]. Forty-six of fifty-five known, unique components were identified in a single, 90 min, on-line, chromatography experiment.

Development of a front-end ETD source for the Orbitrap Velos Pro™ was reported in 2013 [1]. This source facilitates multiple fills of the C-trap with product ions from ETD of intact proteins prior to mass analysis. The result is a dramatic enhancement of the observed ion current without the need for time consuming averaging of data from multiple mass measurements. Here we show that ion–ion proton transfer (IIPT) reactions can be used to simplify ETD spectra and to disperse fragment ions over the entire mass range in a controlled manner. We also show that protein derivatization can be employed to selectively enhance the sequence information observed at the N- and C-termini of a protein.

2. Materials and methods

2.1. Materials

All reagents, not otherwise identified, were purchased from Sigma–Aldrich (St. Louis, MO). Burdick and Jackson® LC–MS grade

Abbreviations: ETD, electron transfer dissociation; IIPT, ion–ion proton transfer.

* Corresponding author at: University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904-4319, USA. Tel.: +1 434 924 3610; fax: +1 434 924 3567.
E-mail addresses: lca4nu@virginia.edu (L.C. Anderson), ame4v@virginia.edu (A. M. English), ww6x@virginia.edu (W.-H. Wang), dlb6z@virginia.edu (D.L. Bai), js4c@virginia.edu (J. Shabanowitz), dfh@virginia.edu (D.F. Hunt).

http://dx.doi.org/10.1016/j.ijms.2014.06.023
1387-3806/

acetonitrile was purchased from Honeywell (Morristown, NJ). Hydrochloric acid, Pierce® LC–MS grade water, LC–MS grade formic acid, DTSSP (3,3′-dithiobis[sulfosuccinimidylpropionate]), phosphate buffered saline (PBS), and urea were purchased from Thermo Fisher Scientific (San Jose, CA). Anhydrous acetyl chloride and methanol were obtained from Alltech Associates, Inc. (Deerfield, IL). Sulfur hexafluoride was purchased as a 10 ppm mixture with nitrogen gas from GTS-Welco (Allentown, PA). Amicon® Ultra-0.5 10 kDa centrifugal filters were obtained from Millipore (Billerica, MA). Fused silica microcapillary tubing was purchased from Polymicro Technologies (Phoenix, AZ).

2.2. Instrument modification

Unless otherwise indicated, experiments were performed on a Thermo Fisher Scientific (San Jose, CA) Orbitrap Velos Pro™. As described recently [1], this instrument was modified to accommodate a glow discharge ion source for generation of negative ions used for electron transfer and proton transfer reactions. Instrument control software (ITCL) was in-house modified to control this ion source discharge, to isolate reagent anions, to enable ion/ion reactions within the QLT using the front-end reagent ion source, and to allow multiple fills of the C-trap with products of these ion/ion reactions.

2.3. Sample preparation

2.3.1. Amidation

Intact apomyoglobin and human CLIP peptide samples were dissolved in a 20 μL solution containing 1 M pyridine, HCl and either 2-(2-aminoethyl) benzimidazole dihydrochloride (intact apomyoglobin) or histamine dihydrochloride (human CLIP peptide), pH ≈ 5.5. A 5-μL aliquot of 0.1 M *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydro-chloride in 1 M pyridine-HCl was added and the solution was sonicated for 2 h. Samples were then taken to dryness in a CentriVap® centrifugal vacuum concentrator (Labconco Corporation, Kansas City, MO). The apomyoglobin sample was reconstituted in 200 μL 0.1% (v/v) acetic acid and desalted using a 10 kDa centrifugal filter prior to direct infusion. Samples were stored at −40 °C until analyzed.

2.3.2. Derivatization with DTSSP and aminoethyl maleimide

Lyophilized apomyoglobin was dissolved in a 100 μL solution containing 6 M urea, 100 mM PBS and 4 mM DTSSP, pH ≈ 7.2 and allowed to cross-link for 2 h at 4 °C. The resulting disulfide bonds were reduced with 160 mM dithiothreitol and alkylated by adding *N*-(2-aminoethyl) maleimide [12] to produce a 320 mM solution and by allowing the reaction to proceed for 15 min at room temperature. The sample was desalted by precipitation with trichloroacetic acid, taken to dryness and stored at −40 °C until analyzed.

2.3.3. Sample preparation for direct infusion

Immediately prior to MS analysis, samples (unmodified and derivatized apomyoglobin) were dissolved in a 200–500 μL solution containing 40% (v/v) acetonitrile and 60%, 0.1% (v/v) acetic acid (v/v). Samples were infused at concentrations of 2–5 pmol/μL.

2.4. MS analyses

2.4.1. Unmodified apomyoglobin

Apomyoglobin (2 pmol/μL) was electrosprayed into the front-end of an ETD/IIPT-enabled Orbitrap Velos Pro™ with the aid of a fused silica, microcapillary column equipped with a laser-pulled (P-2000 microcapillary laser puller, Sutter Instrument Co., Novato, CA), electrospray emitter tip.

Mass analysis was performed by targeting $[M+26H]^{26+}$ ions in a 6 *m*/*z* window centered at *m*/*z* 653. High resolution MS/MS (100,000 at 400 *m*/*z*) spectra were acquired over a mass range of 200–4000 Da and involved collecting 30 multiple C-trap fills of ions generated from 5 ms of ETD and 20–160 ms of IIPT.

2.4.2. Histamine derivatized human CLIP peptide

Human CLIP peptide (250 fmol) in 20 μl 0.1% (v/v) acetic acid was pressure loaded onto a 360-μm o.d. × 75-μm i.d. fused-silica

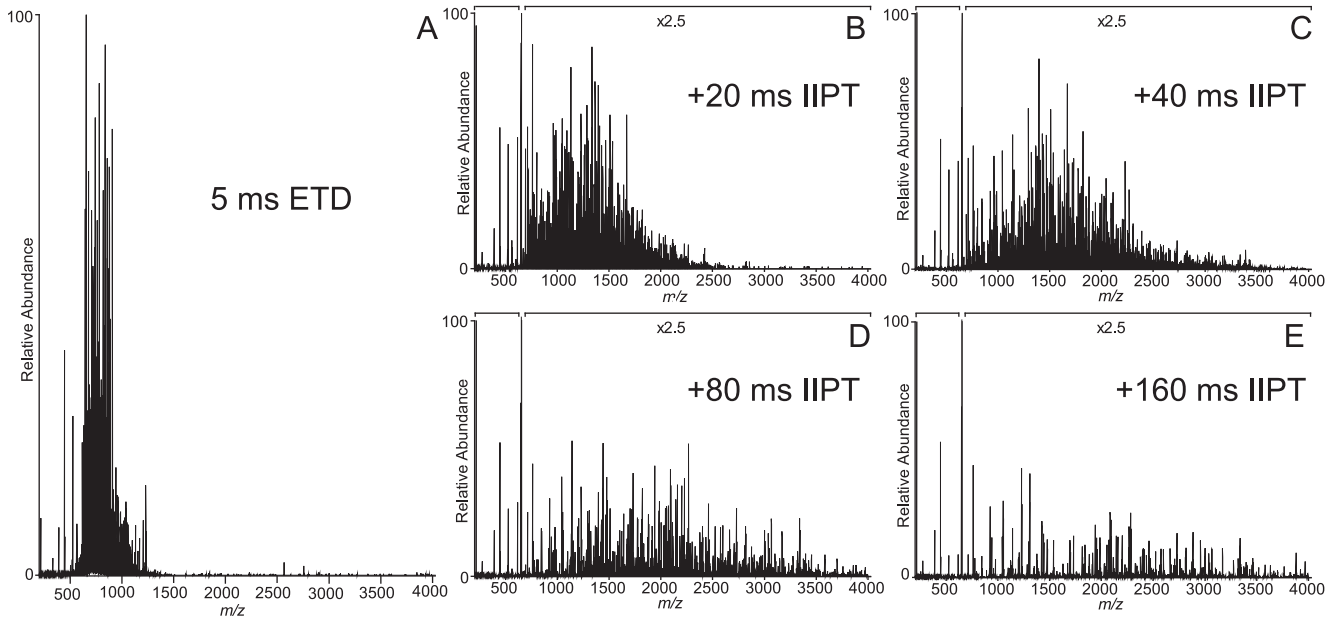

Fig. 1. ETD/IIPT MS/MS spectra recorded on intact apomyoglobin. (A) ETD spectrum recorded on $[M+26H]^{26+}$ ions (*m*/*z* 653) from apomyoglobin using a reaction time of 5 ms. (B–E) Spectra obtained by performing IIPT reactions on the ETD fragment ions in (A) for 20 ms, 40 ms, 80 ms, and 160 ms, respectively.

Please cite this article in press as: L.C. Anderson, et al., Protein derivatization and sequential ion/ion reactions to enhance sequence coverage produced by electron transfer dissociation mass spectrometry, Int. J. Mass Spectrom. (2014), http://dx.doi.org/10.1016/j.ijms.2014.06.023