International Journal of Mass Spectrometry 276 (2008) 144–148

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Characterization and optimization of electron detachment dissociation Fourier transform ion cyclotron resonance mass spectrometry

Jiong Yang, Kristina Håkansson*

Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, MI 48109-1055, United States

ARTICLE INFO

Article history:
Received 25 March 2008
Received in revised form 24 May 2008
Accepted 27 May 2008
Available online 3 June 2008

Keywords:
Electron detachment dissociation
Fourier transform ion cyclotron resonance (FTICR)
Charge state
Oligonucleotide
Nucleic acids

ABSTRACT

We have demonstrated that electron detachment dissociation (EDD) can provide extensive oligonucleotide backbone fragmentation, complementary to that of other MS/MS techniques. In addition, we have shown that, for oligosaccharides, EDD provides additional cross-ring fragments compared to collision-activated dissociation and infrared multiphoton dissociation. In our EDD implementation, the potential difference between a hollow cathode electron source and an extraction lens located in between the cathode and the ion cyclotron resonance (ICR) cell was crucial for successful fragmentation with changes as small as 0.2 V drastically altering fragmentation efficiency, a behavior that was not fully understood. Here, we present a detailed characterization of the electron current passing through the ICR cell as a function of this potential difference, the cathode bias voltage, extraction lens voltage, and the cathode heating current under EDD conditions. Our results show that the extraction lens voltage serves to regulate the number of electrons passing through the ICR cell. Thus, similar electron numbers passing through the cell can be obtained at low (1.2 A) and high (1.8 A) heating current as well as at different cathode bias voltages by adjusting the extraction lens voltage. This characteristic allowed us to investigate the influence of electron energy at fixed electron number and we found that optimum EDD efficiency was obtained with 16–22 eV electrons. We also investigated the influence of charge state on oligonucleotide EDD efficiency and sequence coverage and found that higher charge states provided improved data for a DNA 10-mer, presumably due to a more extended gas-phase structure.

1. Introduction

Tandem Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS/MS) is widely used for biomolecular structural characterization [1–4]. Since the introduction of electron capture dissociation (ECD) in 1998 [5], many research groups have shown that ECD can provide unique fragmentation patterns for molecules as diverse as peptides and proteins [6], nucleic acids [7], polymers [8], lantibiotics [9], and siderophores [10]. ECD involves radical ion chemistry, resulting in more extensive peptide sequence coverage and retention of labile posttranslational modifications [11–13]. In 2001, Zubarev and coworkers introduced a new ion-electron reaction-based fragmentation method operating in negative ion mode; electron detachment dissociation (EDD) [14]. This technique provides unique fragmentation pathways for peptide dianions, including predominant C_α–C backbone bond cleavage. Preferential cleavage of such backbone bonds over side-chain loss in EDD of peptides has been confirmed by ab initio calculations [15]. We have extended EDD to oligonucleotide characterization and demonstrated extensive backbone fragmentation of oligodeoxy- [16] and oligoribo-nucleotides [17], complementary to that of other MS/MS techniques, such as collision-activated dissociation (CAD) and infrared multiphoton dissociation (IRMPD). Our group also showed that EDD can preferentially cleave C–S and S–S bonds in multiply charged disulfide-bonded peptide anions [18], retain higher order structure of DNA hairpins [19], and provide complementary cross-ring fragments for neutral and sialylated oligosaccharides [20]. Fabris and co-workers have applied EDD to oligonucleotide characterization and observed more extensive fragmentation compared to ECD [21]. Furthermore, Amster and coworkers found that EDD produces information-rich tandem mass spectra for glycosaminoglycans, including both cross-ring and glycosidic cleavage product ions [22]. The same group used EDD to distinguish the epimers glucuronic acid and iduronic acid in heparan sulfate tetrasaccharides based on diagnostic product ions, which are not observed in CAD or IRMPD [23].

In our previous EDD implementation [18] on a 7-T Bruker quadrupole (Q)-FTICR mass spectrometer equipped with an indirectly heated hollow cathode electron source [24], optimum fragmentation efficiency was observed at ∼−18 V cathode bias

* Corresponding author. Tel.: +1 734 615 0570; fax: +1 734 647 4865.
E-mail address: kicki@umich.edu (K. Håkansson).

1387-3806/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2008.05.036

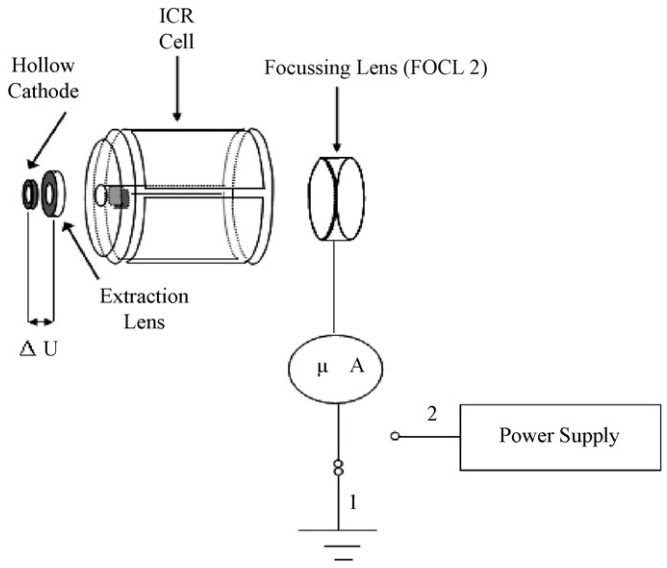

Fig. 1. Experimental configuration for electron current and energy measurements. The microammeter was connected to ground (1) when measuring electron current through the ICR cell, and connected to a floating power supply (2) when measuring the electron energy distribution.

voltage, an extraction lens voltage of ∼−19 V, an irradiation time of 2 s, and a cathode heating current of 1.8 A (see Fig. 1 for a schematic drawing of this set-up). We found that precise tuning of the potential difference (ΔU) between the cathode and the extraction lens was crucial for successful EDD with an optimum around 1 V at 1.8 A heating current, which is the standard heating current used for ECD with the same instrument. Changes of ΔU as small as 0.2 V drastically altered the EDD fragmentation efficiency, a behavior that we did not fully understand at that time. The more negative voltage on the extraction lens compared to the cathode at optimum EDD conditions suggests that so many electrons are emitted that space charge causes them to overcome this small retarding potential. Here, we present a detailed characterization of the electron current passing through the ICR cell as a function of the cathode bias voltage, extraction lens voltage, and cathode heating current in EDD. We also show characterization of EDD efficiency and sequence coverage as functions of precursor ion charge state and electron energy (the latter experiment being greatly facilitated by the insights gained from electron current measurements). Related experiments employing a different set-up for measuring electron current are presented by Amster and co-workers elsewhere in this issue [25].

2. Methods

2.1. Sample preparation

Reversed phase high performance liquid chromatography purified dA_6, dC_6, dT_6, and d(CTATCAGTGA) oligonucleotide ammonium salts were purchased from TriLink BioTechnologies Inc. (San Diego, CA) and the peptide substance P (H-RPKPQQFFGLM-NH_2) was from Sigma (St. Louis, MO). Negative ion mode electrospray solvent consisted of 1:1 (v/v) isopropanol:water (Fisher, Fair Lawn, NJ) with 10 mM ammonium acetate (Fisher). The final concentration of samples was 2–20 μM.

2.2. Fourier transform ion cyclotron resonance mass spectrometry

All experiments were performed with a 7-T Q-FTICR mass spectrometer (Bruker Daltonics, Billerica, MA), which has been previously described [16]. The electrospray source was recently upgraded to include dual ion funnels (Apollo II electrospray ionization (ESI) source, Bruker Daltonics). For EDD fragmentation efficiency characterization, experiments investigating the role of precursor ion charge state were performed with the old ESI source (Apollo I, Bruker Daltonics) whereas all other experiments were performed with the new dual ion funnel source. The ESI flow rate was 70 μL/h in both cases. EDD was performed with an indirectly heated hollow dispenser cathode electron source (Heat Wave, Watsonville, CA). The heater was set to approximately 8.5 V, generating a heating current of 1.8 A, unless specified otherwise. All mass spectra were acquired with XMASS (version 7.0.6, Bruker Daltonics) in broadband mode with 256 or 512k data points and summed over 20–30 scans. Data processing was performed with the MIDAS analysis software [26]: A Hanning window function was applied and data sets were zero filled once prior to fast Fourier transformation followed by magnitude calculation. Peak lists were generated and exported to Microsoft Excel for internal frequency-to-mass calibration with a two-term calibration equation. The calculated masses of the precursor ions and the charge-reduced species were used for calibration. Only assignments better than 20 ppm were included. EDD efficiency calculations were performed by dividing the total product ion abundance with the abundance of precursor ions prior to fragmentation. All abundances were normalized to their associated charge.

2.3. Electron current and energy measurements

Electron current and energy measurements were performed by measuring the electron current impinging on a floating cylindrical focusing element on the opposite side of the ICR cell, just outside the magnetic field (FOCL 2, see Fig. 1) with a digital multimeter (John Fluke, Everett, MA). For non-energy distribution measurements, the focusing element was grounded. An increase of the floating voltage to 20 V did not result in a significant current change thus, we believe that electrons were efficiently collected. The floating voltage necessary for performing the latter experiment, and for measuring energy distributions, was generated by a DC power supply (Goodwill Instrument, Taipei, Taiwan). Electron energy distributions were obtained by derivatizing the electron current with respect to the floating voltage.

3. Results and discussion

3.1. Electron current as a function of cathode bias voltage and extraction lens voltage

Our first series of experiments involved measurements of electron current passing through the ICR cell as a function of cathode bias voltage at fixed ΔU (equal to 1 V). These experiments were motivated by our observation that EDD fragmentation efficiency and fragmentation pattern changed dramatically if the cathode bias voltage was increased from −18 (the experimentally determined optimum) to −30 V at fixed irradiation time and ΔU. At higher cathode bias voltage, precursor ions were almost completely depleted but no product ions were observed. One possible explanation for this behavior is that product ions are too energetic (due to more energetic electrons) and are either ejected from the cell, or further fragmenting. However, an alternative explanation may be that there is a change in electron number as well as electron energy. Injection