International Journal of Mass Spectrometry 378 (2015) 303–311

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Electron transfer dissociation of sodium cationized polyesters: Reaction time effects and combination with collisional activation and ion mobility separation

Bryan C. Katzenmeyer[a,1], Lydia R. Cool[a], Jonathan P. Williams[b], Kirsten Craven[b], Jeffery M. Brown[b], Chrys Wesdemiotis[a,*]

[a] Department of Chemistry, The University of Akron, Akron, OH 44325, USA
[b] The Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, Cheshire SK9 4AX, UK

ARTICLE INFO

Article history:
Received 30 May 2014
Received in revised form
26 September 2014
Accepted 29 September 2014
Available online 13 October 2014

Keywords:
Polyesters
Polylactide
ETD
ETD coupled with CAD
IM–MS

ABSTRACT

The electron transfer dissociation (ETD) characteristics of doubly sodiated polylactide were investigated at varying ion–ion reaction times, ranging from 20 to 220 ms. ETD product ion yields and signal-to-noise ratio maximized at the shortest reaction time. At longer times, the extent of Na/H or H/Na exchange reactions rose, causing spectral crowding; in addition, the sensitivity decreased significantly due to ion losses from neutralization and scattering. ETD in quadrupole ion trap (QIT) and quadrupole/time-of-flight (Q/ToF) mass spectrometers using fluoranthene and *p*-nitrotoluene reagent anions, respectively, led to similar product ions. The Q/ToF configuration allowed for collisionally activated dissociation (CAD) of the ETD total ion current under mild activation conditions, which gave rise to new and more abundant fragment distributions by mainly depleting residual reduced precursor ions. The ETD and ETD/CAD fragmentation patterns were markedly different from the fragments generated by simple CAD, thereby providing complementary structural information about the analyzed polyester. The collision cross-sections of the major ETD fragment series, determined by ion mobility mass spectrometry (IM–MS) experiments on the Q/ToF instrument, agreed well with the linear architectures expected from radical-induced cleavages at the (CO)-O-alkyl bonds promoted by the electron added in the ETD event.

1. Introduction

Several activation methods are available to cause precursor ion fragmentation in tandem mass spectrometry (MS^2) experiments [1]. The most widely used technique in synthetic polymer analyses has undoubtedly been collisionally activated dissociation (CAD) [2,3]. We recently reported the first MS^2 study using electron transfer dissociation (ETD), which was applied to the characterization of biodegradable polyesters [4]. ETD employs ion–ion reactions between a negatively charged reagent anion and a multiply charged precursor cation to energetically excite the precursor so that it decomposes into structurally indicative fragments [5–9]. Our study, conducted on a quadrupole ion trap (QIT) mass spectrometer, showed that ETD of metal-cationized polyesters results in both radical-induced reactions that cleave the COO—alkyl bonds as well as charge-induced reactions that cleave the CO—O bonds [4], ultimately leading to more types of fragments than CAD, which mainly proceeds via charge-remote 1,5-H rearrangements that cleave the COO—alkyl bonds [4,10–12]. Meanwhile, the extent of consecutive fragmentations, which may compromise end group determination, was negligible in ETD, but significant in CAD [4]. These features indicate that ETD could develop into a promising MS^2 method, complementary to CAD, for the determination of polyester structures [4,13], as is true for many types of biopolymers [14–18].

Here, we present a new ETD study, focusing on polylactide, which examines the influence of ion–ion reaction time on the ensuing ETD fragment ion distributions and also compares ETD on a QIT versus a quadrupole/time-of-flight (Q/ToF) instrument equipped with the ion mobility mass spectrometry (IM–MS) [19–21] functionality. IM spectrometry can be viewed as a post-ionization gas-phase separation technique, in which ions travel in an electric field in the presence of a bath gas and, in this process, are dispersed according to their mobilities, which depend on their mass (composition), charge state and collision cross-section (CCS or Ω).

---

[*] Corresponding author. Tel.: +1 330 972 7699; fax: +1 330 972 6085.
E-mail address: wesdemiotis@uakron.edu (C. Wesdemiotis).

[1] Current address: The Valspar Corporation, 2001 Tracy Street, Pittsburgh, PA 15233, USA.

http://dx.doi.org/10.1016/j.ijms.2014.09.021
1387-3806/© 2014 Elsevier B.V. All rights reserved.

The drift time of an ion through the IM region is equivalent to a chromatographic retention time and can be used to determine the corresponding collision cross-section; the latter quantity represents a structurally diagnostic physical constant that reflects ion size and architecture (shape) [19–25].

IM–MS interfaces the IM dimension with mass analysis of the dispersed ions [19]. Several IM–MS methods exist based on the pressure of the bath gas and the type and magnitude of the applied electric field [20]. The traveling wave variant of IM–MS, which employs a pulsed electric field and a relatively low-pressure bath gas [26], was utilized in this study to measure the collision cross-sections and characterize the architectures of polylactide ETD fragments; such insight helps to confirm the proposed fragmentation pathway(s) and deduce with confidence the structure of the polyester precursor ion. This strategy complements a previously developed approach, in which collision cross-sections were acquired for polymeric precursor ions in order to explain the fragment distributions in their MS^2 spectra [27,28].

2. Experimental

2.1. Chemicals

LC–MS grade water, methanol (MeOH) and tetrahydrofuran (THF) were purchased from Fisher Scientific (Pittsburgh, PA). Sodium trifluoroacetate, sodium formate, formic acid (Chromasolv grade), polyalanine and substance P were purchased from Sigma–Aldrich (St. Louis, MO). The polylactide sample was purchased from Polymer Source, Inc. (Dorval, Montreal, Quebec). All chemicals were used without any further purification.

2.2. Sample preparation

A polylactide stock solution with a concentration of 2 mg/mL was prepared in THF. Samples of this solution were directly infused into the mass spectrometers using a syringe pump; the infused samples were prepared by mixing 400 μL of the polymer stock solution with 400 μL of MeOH/THF (1:1, v/v) and 200 μL of a 1-mg/mL MeOH solution of a sodium salt (sodium trifluoroacetate or sodium formate). The concentration of the infused polylactide solution was 0.8 mg/mL.

2.3. ETD-MS^2 and ETD-IM–MS^2 experiments

ETD experiments were performed on QIT and Q/ToF tandem mass spectrometers using $[M+2Na]^{2+}$ precursor ions formed by electrospray ionization (ESI). With both instruments, ESI conditions and sample infusion rates were optimized to maximize the intensity of $[M+2Na]^{2+}$ and to limit in-source collisional activation and fragmentation.

The QIT was a Bruker HCT Ultra II ion trap (Bruker Daltronics, Billerica, MA) [4,29]. With this instrument, the samples were infused into the ESI source at a flow rate of 3 μL/min. The tip of the ESI needle was grounded and the entrance of the capillary, through which the ions enter the vacuum system of the QIT, was held at −3.5 kV. The pressure of the nebulizing gas (nitrogen) was set at 10 psi and the flow and temperature of the drying gas (nitrogen) at 8 L/min and 300 °C, respectively. Fluoranthene radical anions (*m/z* 202), which served as the ETD reagent, were produced in a negative chemical ionization (nCI) source filled with methane buffer gas (2.0–2.6 bar). The nCI source is located above the octapole lens that transfers ESI-generated ions to the ion trap; it was operated using an electron energy of 70 eV, emission current of 2 μA and reactant remove cutoff of *m/z* 210. A gate lens at the exit of the nCI source is used to transfer either the precursor cations or reagent anions into the ion trap. After accumulation of both types of species in the trap, the ion–ion reaction time was varied between 20 and 300 ms to determine the effect of this variable on the ETD fragment ions observed. Data acquisition and processing were carried out using the Bruker Esquire-Control program (version 6.2).

Experiments were also performed on a hybrid quadrupole/ion mobility/orthogonal acceleration ToF mass spectrometer (Synapt-G2, Waters Corporation, Manchester, UK) [26,30] fitted with the ETD functionality. The instrument was operated with an ESI capillary voltage of +3.4 kV and a sample cone voltage of +30 V. For ETD-type fragmentation, a sub-ambient pressure (~2 mbar) glow discharge source was used, which has been described in detail elsewhere [31]. This instrument contains three consecutive, gas filled, traveling wave (T-Wave) RF stacked ring ion guides between the Q and ToF mass analyzers, called trap cell (closest to Q), ion mobility (IM) cell and transfer cell (closest to ToF). ETD spectra were measured by using the glow discharge anion source to populate the trap T-Wave cell with mass selected ETD reagent anions formed from *p*-nitrotoluene (*m/z* 137). For this, a discharge voltage of −300 V was applied to the glow discharge electrode relative to the sample cone voltage; the resulting discharge current was ~35 μA. Reagent anion fill times were typically 100 ms. The source polarity and quadrupole set mass were subsequently switched to allow $[M+2Na]^{2+}$ cations formed by ESI of polylactide to enter the trap cell and interact with stored reagent anions for approximately 20 ms [31]. This interaction enables ion–ion type reactions resulting in ETD product ions which were separated according to their ion mobilities in the IM cell. Upon exiting the latter cell, the ions traverse the transfer T-Wave cell which directs them into the ToF analyzer and can optionally provide supplemental activation (CAD) prior to the ToF mass analysis. For efficient ETD, the bath gas used in the trap T-Wave cell was helium set to a pressure of 5×10^{-2} mbar. The transfer T-Wave cell was pressurized to 5×10^{-3} mbar with argon. The trap T-Wave velocity and amplitude, which influence the ion–ion interaction time as well as the reaction rate, were set to 300 m/s and 0.3 V, respectively. The IM T-Wave cell was operated at 3.3 mbar of nitrogen with a T-wave velocity and pulse amplitude of 650 m/s and 25 V, respectively. For combined ETD/CAD spectra, the bias of the transfer cell was adjusted to 15 V, whereas conventional CAD spectra were acquired using the transfer cell at a bias of 30 V. Such voltage settings have been used in similar experiments on doubly protonated peptides [32]. For the ETD/CAD data, the T-wave amplitude in the trap cell (location of the ion–ion reaction) and the bias of the transfer cell (location of supplemental CAD) were set at values (vide supra) that balanced the ETD and CAD yields, so that both processes contributed to the observed fragments. Data acquisition and processing were carried out using the Waters MassLynx (version 4.1) and Driftscope (version 2.1) programs. Polylactide was infused into the ESI source at a flow rate of 5 μL/min.

2.4. Instrument calibration for the acquisition of collision cross-sections

The collision cross-sections (CCS or Ω) of the ETD product ions were derived from the corresponding measured drift times (t_d) by calibrating the T-Wave mobility device with ionic species of known Ω [33–35]. Calibrants were the $[M+H]^+$ ions of polyalanine oligomers whose Ω values had been previously established by standard DC-drift tube IM–MS experiments [36,37]. Polyalanine was ionized by ESI of a 1-mg/mL solution in water/MeOH (1:1, v/v) and the drift times of $[\mathrm{Ala}_n+H]^+$ ($n = 3$–19; *m/z* 232.1–1368.7) were used to construct a calibration curve over the Ω range 89.0–276.3 Å^2. For this, the normalized CCS of each oligomer, $\Omega' = \Omega_{\text{published}} \times (\mu^{0.5}/z)$ (μ = reduced mass of ion/drift gas complex; z = ion charge), was plotted against the corresponding corrected drift time, $t'_d = t_d - [C \times (m/z)^{0.5}/1000]$ (where C is a correction factor for instrumental flight time offsets) [33]. Power