International Journal of Mass Spectrometry 283 (2009) 85–93

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Electron transfer dissociation of oligonucleotide cations

Suncerae I. Smith, Jennifer S. Brodbelt*

Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712, United States

ARTICLE INFO

Article history:
Received 2 December 2008
Received in revised form 12 January 2009
Accepted 4 February 2009
Available online 24 February 2009

Keywords:
Oligonucleotide
Electron transfer dissociation
Collision induced dissociation
Linear ion trap

ABSTRACT

Electron transfer dissociation (ETD) of multi-protonated 6–20-mer oligonucleotides and 12- and 14-mer duplexes is compared to collision activated dissociation (CAD). ETD causes efficient charge reduction of the multi-protonated oligonucleotides in addition to limited backbone cleavages to yield sequence ions of low abundance. Subsequent CAD of the charge-reduced oligonucleotides formed upon electron transfer, in a net process termed electron transfer collision activated dissociation (ETcaD), results in rich fragmentation in terms of *w*, *a*, *z*, and *d* products, with a marked decrease in the abundance of base loss ions and internal fragments. Complete sequencing was possible for nearly all oligonucleotides studied. ETcaD of an oligonucleotide duplex resulted in specific backbone cleavages, with conservation of weaker non-covalent bonds.

1. Introduction

There is ongoing interest in exploring alternative electron-based ion activation/dissociation methods, such as electron capture dissociation (ECD) [1,2], electron detachment dissociation (EDD) [3,4], and ion–ion reactions [5–13], for sequencing nucleic acids. Collision activated dissociation (CAD) of deprotonated oligonucleotides is initiated by loss of a neutral or charged base, followed by subsequent backbone fragmentation into complementary *w* and *a*–*B* ions [14] (see Scheme 1). In contrast, the dissociation of radical oligonucleotide anions, first illustrated by McLuckey et al. [7] through ion–ion reactions, provides additional and complementary information to that afforded by CAD, with a significant decrease in base loss ions and substantial formation of *w* and *a* ions. For ion–ion reactions of oligonucleotide anions, the majority of reactants studied, including benzoquinoline and $C_4H_9^+$ [5], protonated pyridine [10,11], trifluoroacetic acid [12], $Xe^{+\bullet}$ [7,13] and CCl_3^+ [7], exclusively promote charge reduction by either proton transfer or electron transfer. In the case of $Xe^{+\bullet}$ and CCl_3^+, CAD of the resulting charge-reduced odd-electron products subsequently resulted in the formation of *w* and *a* ions with no observable base loss [7]. Backbone fragmentation was observed upon reaction of oligonucleotide anions with $O_2^{+\bullet}$ [5] and Ar^+, Kr^+, Xe^+ [13], and the degree of fragmentation was found to be related to the exothermicity of the ion–ion reaction [5].

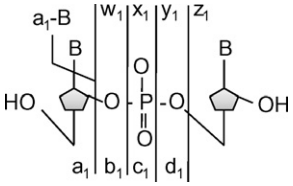
ECD of protonated oligonucleotides generates radical cations and a complex array of low abundance product ions, including *w*/*d* ions, *a*/*z* ions, *c*/*x* ions, and both $wH/dH^{+\bullet}$ and $aH/zH^{+\bullet}$ radical ions, in addition to those that entail base loss from many of the fragment ions (*w*/*d* − *B*, *c*/*x*–*B*, *a*/*x*–*B*), a feature which complicates spectral interpretation [1]. (In palindromic sequences, *w* and *d*, *a* and *z*, and *c* and *x* ions have the same *m*/*z* values.) For the non-palindromic sequence dGCATGC, one $zH^{+\bullet}$ and one $wH^{+\bullet}$ ions were observed [1]. Despite the significant number of product ions observed, sequence coverage was extensive but not complete for several of the six-mers studied [1,2].

EDD of oligonucleotide anions generates radical and non-radical products similar to those obtained by ECD [3,4]. Upon EDD, *w*/*d* and $a^{\bullet}/z^{\bullet}$ series were observed for palindromic oligonucleotide sequences, and one $z^{\bullet}$ ion was observed for the non-palindromic sequence dGCATGC [4]. EDD of a small (6-mer) oligonucleotide duplex [4] and several large (15-mer) oligonucleotide hairpins [3] did not result in disruption of the fragile non-covalent interactions of the base pairs.

Because near-thermal electrons cannot be stabilized simultaneously with analyte ions of interest in ion traps that use radio frequency electrostatic fields, molecular anions were developed as vehicles for delivering electrons to multiply charged peptide and protein cations, an ion activation process termed electron transfer dissociation (ETD) [15]. Upon electron transfer, which is typically initiated by using the fluoranthene radical anion as an electron donor, the resulting charge-reduced peptides, which are radical cations and no longer even-electron ions, are unstable and undergo subsequent dissociation. The process of ETD in linear ion traps is similar to ECD in FTICR mass spectrometers [15–17]. Like ECD, ETD provides a significant increase in sequence coverage for highly charged peptides (charge >3) [16], and several methods have been implemented to increase ETD efficiency for doubly protonated

* Corresponding author. Tel.: +1 512 471 0028; fax: +1 512 471 8696.
E-mail address: jbrodbelt@mail.utexas.edu (J.S. Brodbelt).

1387-3806/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2009.02.012



Scheme 1. Oligonucleotide fragmentation nomenclature.

peptides [18,19]. ETD has not yet been studied for oligonucleotide cations, which is the focus of the present study; however, related work by McLuckey et al. has entailed the exploration of several ion–ion reactions of positively charged nucleic acids. For protonated oligonucleotides, reactions with perfluorocarbon anions results exclusively in charge reduction by proton transfer [6,8]. No backbone fragmentation was observed.

Another electron-based ion activation method compatible with ion traps is electron photodetachment dissociation, EPD [20,21]. In this process, oligonucleotide anions are irradiated with 260 nm photons, and then the resulting electron-detached species are subjected to CAD. In addition to neutral losses, a large number of products identified as *w*, *d*, $a^{\bullet}$, and $z^{\bullet}$ ions were observed. Good sequence coverage was obtained for single strand oligonucleotides up to 20 nucleotides in length for this technique [20,21].

In the present study, we explore the fragmentation patterns of positively charged oligonucleotides, both single strands and duplexes, using ETD. In all cases, ETD causes efficient charge reduction of the multi-protonated oligonucleotide cations, producing oligonucleotide radical cations. Subsequent CAD of these charge-reduced oligonucleotide radical ions, in a net process termed electron transfer collision activated dissociation (ETcaD) [19], results in backbone fragmentation which is more extensive than that promoted by CAD of the corresponding even-electron species.

2. Experimental

2.1. Chemicals

The following oligodeoxynucleotides were obtained from Integrated DNA Technologies (Coralville, IA) on the 1.0 μM scale and used without further purification: 5′-AAAAAA-3′, 5′-CCCCCC-3′, 5′-GGGGGG-3′, 5′-TTTTTT-3′, 5′-TGGCCA-3′, 5′-ATGACTCG-3′, 5′-GTATGACTCGCA-3′, 5′-TCGTATGACTCGCAAG-3′, 5′-CATCGTATGACTCGCAAGTG-3′, 5′-GCGGGGATGGGGCG-3′, 5′-CGCCCCATCCCCGC-3′, 5′-CCCGGGTTTAAA-3′, and 5′-ATGCTGCCCGGG-3′. Oligonucleotide single strand concentrations were determined spectrophotometrically by Beer's Law using the extinction coefficients provided by the manufacturer. Annealing was performed by preparing stock solutions containing 2 mM of each complementary ODN in 100 mM ammonium acetate. The solutions were heated to 90 °C for 10 min and slowly cooled to room temperature overnight. The following duplexes were created: duplex 1 = 5′-GCGGGGATGGGGCG-3′/5′-CGCCCCATCCCCGC-3′ and duplex 2 = 5′-CCCGGGTTTAAA-3′/5′-ATGCTGCCCGGG-3′. For ESI-MS analysis, the solution was diluted to 10 μM of oligonucleotide by preparing in 20 mM ammonium acetate solution.

2.2. Mass spectrometry

Oligonucleotide samples were directly electrosprayed into a Finnigan LTQ mass spectrometer (Thermo Electron Corp., San Jose, CA). A Harvard syringe pump (Holliston, MA) at a flow rate of 3 μL/min was used. The ESI source was operated in the positive ion mode with an electrospray voltage of 4.0 kV and a heated capillary temperature of 90 °C. To assist in desolvation, nitrogen sheath and auxiliary gas were applied at 40 and 20 arbitrary units, respectively. Spectra were acquired by summing 20 scans.

In the CAD experiments, collisional activation voltages were applied at a level required to reduce the isolated precursor ion to ~10–20% of its original abundance. The default activation time of 30 ms was used in all CAD experiments with a q_z value of 0.25. ETD reactions were performed with radical anions of fluoranthene generated by a chemical ionization source, typically for 100 ms unless otherwise noted. For ETcaD experiments, electron transfer was performed for typically 100 ms, and collisional activation voltages were again applied at a level required to reduce the isolated precursor ion to ~10–20% of its original abundance. The default activation time of 30 ms was used in all ETcaD experiments, and the q_z value was set to 0.25 except for the experiments in which q_z was varied. The isolation width was set to 5 *m*/*z* for all MS/MS steps.

3. Results and discussion

3.1. Positively charged oligonucleotides

Most previous ESI-MS investigations of DNA oligonucleotides and duplexes have been carried out using the negative ion mode. This follows logically from the knowledge that the phosphodiester backbone of the oligonucleotide has a $pK_a < 1$, and is therefore fully deprotonated under most experimental conditions. In order to observe protonated nucleic acids, all the phosphates must be protonated, and several extra ionizing protons must also be added, most likely initially localized at the nucleobases [22]. A handful of ESI-MS studies have been carried out on positively charged DNA oligonucleotides [23–27], and most authors concede that analysis is often easier in the negative mode, i.e., higher signal and a decrease in salt adducts are typical in the negative ion mode. Despite the generally accepted benefits of the negative mode for oligonucleotide detection by mass spectrometry, analysis in conjunction with ETD (or ECD) is not feasible for negatively charged precursor ions which motivated us to assess the positive mode in order to exploit ETD for structural characterization of oligonucleotides.

Using the LTQ linear ion trap instrument, oligonucleotide ions are easily produced in the negative or positive mode upon ESI (Fig. 1). For most oligonucleotides (dA_6, dC_6, dG_6, ss6, ss8–ss20 (Table 1)), the total ion abundance is greater in the positive mode, and similar spectral quality is observed for both ion polarities (Fig. 1a and b). Due to the low gas-phase basicity of thymine, molecular ions of dT_6 are not expected to form in the positive ion mode [19]. However, dT_6 was easily observed in the positive mode in the linear ion trap, although the ion abundance is slightly lower than in the negative mode (Fig. 1c and d). In addition, a slight shift to higher charge states is observed for most oligonucleotides in the positive mode, with the exception of dT_6, in which the doubly charged ion is most abundant in both the positive and negative modes.

Table 1
Summary of the oligonucleotide sequences used in this study.

Name	Sequence	Molecular weight (g/mol)
dA_6	5′-AAAAAA-3′	1817.3
dC_6	5′-CCCCCC-3′	1673.1
dG_6	5′-GGGGGG-3′	1913.3
dT_6	5′-TTTTTT-3′	1763.2
ss6	5′-TGGCCA-3′	1792.2
ss8	5′-ATGACTCG-3′	2409.6
ss12	5′-GTATGACTCGCA-3′	3645.4
ss16	5′-TCGTATGACTCGCAAG-3′	4881.2
ss20	5′-CATCGTATGACTCGCAAGTG-3′	6117.0
d_1	5′-GCGGGGATGGGGCG-3′/5′-CGCCCCATCCCCGC-3′	8531.6
d_2	5′-CCCGGGTTTAAA-3′/5′-ATGCTGCCCGGG-3′	7307.8