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Ion/ion reactions of multiply charged nucleic acid anions: electron transfer, proton transfer, and ion attachment

Jin Wu, Scott A. McLuckey*

Department of Chemistry, Purdue University, West Lafayette, IN 47907-2084, USA

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In honor of Helmut Schwarz for his many contributions to mass spectrometry in particular and to science in general.

Abstract

Charge transfer reactions, including electron transfer, proton transfer, and cation attachment, have been investigated for ion/ion reactions involving multiply charged oligonucleotide anions. Low-mass cations, such as the oxygen cation ($O_2^{\bullet+}$) and protonated isobutylene ($C_4H_9^+$), have been used to manipulate charge states of high-mass oligonucleotide anions with the "trapping by proxy" approach in a quadrupole ion trap. Oxygen cations react with oligonucleotide anions by electron transfer reactions, inducing a series of w- (or d-), (a-B)-, and z-type fragment ions for polydeoxyadenylate (polydA) and polydC anions. Much less fragmentation is noted for anions of polydT and 5′-d$(CGGG)_5$-3′. Protonated isobutylene, $C_4H_9^+$, undergoes proton transfer reactions with oligonucleotide anions with significantly less fragmentation. Protonated benzoquinoline (BQ) ions react with oligonucleotides predominantly by proton transfer with minimal fragmentation, but show a small degree of attachment to polydT and G-rich oligonucleotides. The extent of fragmentation associated with ion/ion reactions involving oligonucleotides appears to be directly related to ion/ion reaction exothermicity. Electron transfer ion/ion reaction products do not appear to be significantly more or less stable than proton transfer products of the same charge state. Protonated BQ, due to its higher mass than isobutene or oxygen, obviates the need for trapping by proxy and allows the ion parking technique to be applied to oligonucleotide anions. Peptide cations react with oligonucleotide anions via proton transfer but show extensive adduct ion formation as well. Neither the N-terminal amino group nor the C-terminal carboxy group appears to be essential in adduct ion formation. Rather, the possibility for multiple non-covalent interactions between polar groups on the peptide and oligonucleotide leads to the formation of stable adducts.

Keywords: Charge transfer; Ion/ion reactions; Oligonucleotide anions; Trapping by proxy; Ion parking

1. Introduction

Electrospray ionization (ESI) [1,2] is one of the soft ionization methods that have been widely used to form gaseous ions from large biomolecules such as peptides, proteins, oligonucleotides, and carbohydrates. The well-known multiple-charging phenomenon associated with ESI has several beneficial consequences including, for example, multiple mass measurements arising from a plurality of peaks for a single compound, compatibility with mass analyzers which have limited mass-to-charge range (e.g., the

* Corresponding author. Tel.: +1-765-494-5270; fax: +1-765-494-0239.
E-mail address: mcluckey@purdue.edu (S.A. McLuckey).

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quadrupole mass filter), and ease of transmission of a mixture of ions with widely different mass and charge but similar mass-to-charge ratio through the atmosphere/vacuum interface [3–5]. However, it is often desirable to be able to manipulate the initial charge distribution. It is useful to do so to facilitate the analysis of complex mixtures [6,7], to form ions that are not directly produced by ESI for subsequent analysis [8–10] and to reduce the product ion charge states to simplify identification of product ions [11–15].

A number of approaches have been employed to reduce charge states of high-mass ions. These include manipulation of solution phase conditions [16,17] and the use of ion chemistry to reduce ion charge in the gas phase. Two distinct approaches to charge transfer in the gas phase have been employed. One approach takes advantage of ion/molecule reactions [18–21] whereas the other exploits ion/ion reactions [22–28]. An ion/molecule reaction strategy for manipulating charge states is relatively straightforward to effect in many types of instruments. It simply requires either the admission of a gaseous base for deprotonation of multiply protonated molecules or the admission of a gaseous acid for protonating multiply deprotonated molecules. However, ion/molecule reactions usually have limited effectiveness in reducing the charge of highly charged macro-molecules to arbitrarily low values. The extent to which charge transfer occurs is dependent on factors such as the basicity or acidity of the neutral molecule, charge and structure of the macro-ion, and the basicities of the charge sites in the macro-ion [29]. Clustering reactions can sometimes compete with proton transfer [18], particularly when the barriers to proton transfer are similar to the barriers for loss of the neutral species from the collision complex. An additional problem for ion trapping instruments is that it is difficult to admit and remove the neutral reagents rapidly enough to prevent charge permutation reactions at inconvenient times, e.g., during ion accumulation and mass analysis. Ion/ion reactions, on the other hand, are more difficult to implement than are ion/molecule reactions but they overcome the disadvantages of ion/molecule reactions just described. For example, the ion/ion reactions for all charge states are highly exothermic such that charge states of biopolymer ions can be readily reduced to either +1 or −1. Clustering reactions have been observed but can be avoided by use of the appropriate ions [23,25,30]. A further advantage of ion/ion reactions for reducing charges is that the injection and removal of ions from a reaction region can be controlled more readily than the admission and removal of neutral species. Ion/ion reactions have been used prior to sampling ions into a mass spectrometer [22,26–28] and within quadrupole ion traps in which ions of opposite polarity can be stored simultaneously and in overlapping regions of space [23–25]. The latter approach maximizes experimental flexibility by enabling the use of ion/ion reactions in conjunction with experiments involving multiple stages of mass spectrometry.

Ion/ion reactions allow for a wide range of charge transfer chemistries to be studied, such as electron transfer, proton transfer, ion attachment, metal ion transfer, and metal attachment. In most mixture analysis scenarios, it is highly preferred that ion/ion reactions lead to a final ion population that is as homogeneous in mass and charge as possible, such as the $[M - H]^-$ or $[M + H]^+$ ions, without giving rise to fragmentation, ion attachment, or an upper limit to the mass-to-charge ratio of the biopolymer ion [7]. Electron transfer reactions usually result in a range of product-ion masses that reflects the range of parent-ion masses subjected to reaction. For instance, the −1 charge state ions arising from a distribution of parent ion charges of 2^-, 3^-, 4^-, and so forth, is a mixture of $[M-2H]^{\bullet -}$, $[M-3H]^{\bullet\bullet -}$, $[M-4H]^{\bullet\bullet\bullet -}$, etc. Sodium transfer also gives rise to a mixture of product ions. In contrast, when the multiply charged ions arise from a distribution of deprotonated species, proton transfer yields the same mass product ion species at every charge state regardless of the initial parent ion charge state. Proton transfer, therefore, is the favored mechanism for charge reduction of negatively charged macro-ions formed via deprotonation. In all cases, fragmentation is undesirable because it can lead to complex spectra that are difficult to interpret [29].

Multiply charged anions derived from oligonucleotides have been subjected both to ion/ion proton transfer [11,23] and electron transfer [24,29,31] reactions. Protonated pyridine has been utilized to determine product ion charge states in ion trap tandem mass spectrometry of poly-anions [11]. Electron transfer reactions with rare gas cations have been observed to result in extensive fragmentation of relatively small oligonucleotides ($n = 3$–5) [24,31] but yield predominantly charge reduction products with minimal fragmentation for a mixed-base 16-mer oligonucleotide [29]. To shed further light onto the gaseous ion/ion chemistry of nucleic acid anions, we examined several charge transfer reactions, including electron transfer, proton transfer, and ion attachment, involving multiply charged homopolymer and mixed-base oligonucleotide anions. Particular emphasis was placed on the objective of establishing reagent ions that can be used to manipulate charge states of large multiply charged nucleic acid anions without complications arising from fragmentation, ion attachment, or limited dynamic range for trapping ions of different mass-to-charge values.

2. Experimental

2.1. Materials

The mixture of 5′-pd$(A)_{40–60}$-3′ was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Homopolymers 5′-d(AAAA)-3′, 5′-d$(A)_{20}$-3′, 5′-d$(C)_{20}$-3′, 5′-d$(T)_{20}$-3′, 5′-d(CGGGCGGGCGGGCGGGCGGG)-3′, and mixed-base 50-mer (5′-d(GGGTCTGATCTTCTACCCGGGCAACTGGCCGATCATCGCGCCGTCGCACG)-3′) were custom synthesized by Integrated DNA Technologies (Coralville, IA). The 12-mer, 5′-d(CTTAGCGCTAAG)-3′, was a gift from Prof. J. Banoub of the Canadian Department of Fisheries and Oceans and the Memorial University of Newfoundland. 5S ribosomal RNA from *E. coli*, 120 bases, was purchased from Sigma (St. Louis, MO).

Isobutane was obtained from BOC Gases (Lebanon, NJ). Leucine enkephalin (LE) was purchased from Sigma. Piperidine, imidazole, benzoquinoline (BQ), HCl, acetyl chloride, ammonium bicarbonate, and acetic anhydride were obtained from Aldrich (Milwaukee, WI). Ultrapure water was provided from a NANOpure Infinity ultrapure water system (Barnstead/Thermolyne Corporation, Dubuque, IA).

2.2. Sample preparation

DNA samples were purified prior to analysis by ethanol precipitation [7,32–34]. Samples were first dissolved in ultrapure water. Precipitation was carried out by adding 10 M aqueous ammonium acetate to a DNA solution to bring the solution to ca. 2.5 M in ammonium acetate, followed by addition of 2.5 volumes of ice-cold ethanol. After incubation at −20 °C for at least 2 h, the DNA pellet was collected by centrifugation at room temperature for 40 min at 10,000 rpm. The supernatant was decanted, and the pellet was dried for 20 min in a centrifugal concentrator (SpeedVac, Savant UVS400, Holbrook, NY). The pellet was washed once with 200 μL of cold ethanol and once with 70% aqueous ethanol by vortexing followed by centrifugation for 20 min at 10,000 rpm, and then dried by SpeedVac. The above procedure was repeated twice. Following ethanol precipitation, the DNA pellet was stored at −20 °C. It was re-suspended in water to a concentration of 100–200 μM as a stock solution just before mass spectrometric analysis.

The methyl ester of LE was prepared by the addition of 250 μL of 2N HCl in methanol, prepared by the dropwise addition of 480 μL of acetyl chloride to 3 mL of anhydrous methanol with stirring, to 2.5 mg of LE. The reaction was allowed to proceed for 2 h at 25 °C. The sample was dried by SpeedVac, followed by purification by HPLC [35].

N-acetylation of LE was performed using the following procedure [35]: 10 mg of LE were dissolved in 100 μL of 50 mM ammonium bicarbonate (pH 7.8). To this solution, 1 mL of the acetylation reagent, prepared by the addition of 250 μL of acetic anhydride to 750 μL methanol, was added and the reaction proceeded for 2 h at 25 °C. The sample was dried by SpeedVac and purified by HPLC.

2.3. Mass spectrometry

DNA solutions for negative nano-electrospray were prepared by diluting the aqueous stock solutions to 8 ∼ 20 μM in 25 mM piperidine and 25 mM imidazole aqueous solution except that 5′-d(AAAA)-3′ was prepared in pure water. Solutions of BQ and LE were nano-electrosprayed in the positive ion mode at a concentration of 1 mg/mL. The nano-electrospray needle was pulled from borosilicate glass capillaries with a 1.5 mm o.d. and a 0.86 mm i.d. using a model P-87 Sutter Instruments micropipet puller (Sutter Instruments, Novato, CA). The electric connection to the solution was effected by inserting a stainless steel wire through the back of the capillary, with typical nano-electrospray potentials of around ±1.2 kV with the polarity of the wire defining the ion polarity.

The experiments with oxygen cation ($O_2^{\bullet+}$) and protonated isobutylene ($C_4H_9^+$) were conducted on a home-built ESI source coupled with a Finnigan MAT (San Jose, CA) ion trap mass spectrometer [36] modified to allow the injection of ions through a 3-mm hole in the ring electrode [30]. An atmospheric sampling glow discharge ionization (ASGDI) source [37] was mounted in-line with the ring electrode hole. Singly charged $O_2^{\bullet+}$ and $C_4H_9^+$ ions used in this work to reduce multiply charged oligonucleotide anions were formed by glow discharge ionization.

To minimize the appearance of cluster ions in the ion population generated by ESI, one or more so-called "heating ramps" [7] were employed to effect declustering in the ion trap. A typical experiment consisted of an oligonucleotide anion accumulation time (200–600 ms), heating ramps (30–60 ms), isolation of the ions of interest (60–120 ms), followed by cation injection (260–400 ms), and final mass analysis using resonance ejection [38]. Specifically, the oxygen ions were continuously injected into the ion trap at a low-mass cutoff (LMCO) of 25 for 280–400 ms. Protonated isobutylene ions were continuously admitted into the ion trap at an LMCO of 35 for 260–320 ms. The LMCO was then immediately raised to at least 150 to eject the low-mass positive ions and to provide a sufficiently deep well depth for trapping the high mass-to-charge ratio oligonucleotide ions arising from ion/ion reactions. For the experiments involving collisional activation, a resonance excitation step of 100–200 ms was added between the ion isolation and the cation injection step.

The experiments involving LE and BQ were carried out on another modified Finnigan MAT ion trap (San Jose, CA) which has been described in detail elsewhere [39]. Briefly, the instrument employs a DC turning quadrupole to sequentially direct the ions of opposite polarities into the ion trap from two electrospray sources that are located 90° from the axial dimension of the trap and 180° away from each other. The oppositely charged ions are simultaneously stored in the ion trap and undergo charge transfer reactions. A typical experiment performed on this instrument involved DNA anion accumulation (100–300 ms), heating ramps (30–60 ms), isolation of selected ions (60–120 ms), cation accumulation (80–500 ms, LMCO of 50 for LE, LMCO of 30 for BQ), mutual storage time for ion/ion reaction (100–500 ms, LMCO 150), ejection of cations (30 ms) to avoid the deleterious effects on mass analysis which can arise due to the presence of ions of opposite polarity [40], and mass analysis by resonance ejection.

In all cases, helium was admitted into the ion trap to a total pressure of 1 mTorr with a background pressure of 2×10^{-5} Torr in the instrument without addition of helium. The spectra derived from normal nano-electrospray are called "pre-ion/ion" mass spectra whereas those recorded after ion/ion reactions with ion charge states reduced are referred to as "post-ion/ion" mass spectra. The spectra shown here are typically the average of 300–600 scans.

2.4. Theoretical calculations

Semi-empirical calculations were performed with Gaussian 98W (Gaussian, Inc., Carnegie, PA) to obtain model structures and energetics for the protonated BQ and deprotonated oligonucleotides. $(CH_3O)_2OPO^-$ was used as a model compound for deprotonated nucleotides. The structures of compounds were optimized at the Hartree–Fock (HF) level using 6-31G*

[41] as the basis set. The optimized structures were then subjected to a single point energy calculation of the correlated energy at the MP2/6-31G* level of theory. Energies were corrected for zero-point vibrations scaled by 0.9135 [42]. The proton affinities were determined from the differences between calculated correlated energies of the geometry optimized neutral molecules and protonated/deprotonated molecules.

3. Results and discussion

Gas-phase electron transfer, proton transfer, and ion/ion combination (cation attachment) have been observed in reactions of multiply charged DNA anions with singly charged cations. The general form of each of these reactions can be written as follows:

Electron transfer

$$[\mathrm{M} - n\mathrm{H}]^{n-} + \mathrm{B}^{\bullet +} \rightarrow [\mathrm{M} - n\mathrm{H}]^{\bullet (n-1)-} + \mathrm{B} \quad (1)$$

Proton transfer

$$[\mathrm{M} - n\mathrm{H}]^{n-} + \mathrm{BH}^{+} \rightarrow [\mathrm{M} - (n-1)\mathrm{H}]^{(n-1)-} + \mathrm{B} \quad (2)$$

Cation attachment

$$[\mathrm{M} - n\mathrm{H}]^{n-} + \mathrm{BH}^{+} \rightarrow [\mathrm{M} - (n-1)\mathrm{H} + \mathrm{B}]^{(n-1)-} \quad (3)$$

A wide variety of reagent species, B, can give rise to radical cations or protonated molecules. In this work, oxygen is used as B for the study of electron transfer, isobutene and BQ are used as B for the study of proton transfer reactions, and LE is used as B for the study of ion attachment.

3.1. Electron transfer

The limit to which charge states can be reduced in a quadrupole ion trap is often determined by the ion trapping conditions under which ions of opposite polarity and widely different mass-to-charge ratios can be stored simultaneously. Typically, ions of both polarities must be stored at q_z values less than 0.908. This constraint can give rise to an upper limit to the mass of the macro-ion that can be reduced in charge to either $+1$ or -1 as a result of weak ion confinement at very low values of q_z. This potential compromise is minimized by the use of high-mass reagents for the manipulation of macro-ion charge states. Relatively high-mass singly charged anions, derived from perfluorocarbons, suitable for reducing positive charges have been identified [25] and have played an important role in the study of positively charged proteins [6,9,10,13,14,43–45]. Perfluorocarbon anions are readily generated in high abundance by glow discharge due to their relatively large electron capture cross-sections and high volatilities. However, an analogous compound class amenable to glow discharge ionization that can be equally effective in manipulating anion charge states is not readily identifiable, primarily due to the low volatilities associated with polar high-mass molecules [7]. Protonated pyridine [11,23] and rare gas cations such as xenon [24,29,31], produced by glow discharge, have been demonstrated to be capable of reducing the charges of multiply charged oligonucleotide anions but such low-mass cations constrain the mass of oligonucleotides amenable to charge state reduction to -1 to roughly 10 kDa or less.

An approach to overcome the limitation associated with the finite mass-to-charge range for mutual ion storage, referred to as “trapping by proxy,” was recently described [7]. This approach relies on the ability to admit an intense beam of positive ions continuously into the ion trap. The electric field generated by a sufficiently large number of stored positive ions can serve to trap high-mass negative ions formed in the ion/ion reaction sequence when the oscillating quadrupolar field is too weak to do so. The ion trap is used to store the positive ions that, in turn, store the negative ions. In this scenario, high-mass negative ions are trapped “by proxy.” For trapping by proxy to be effective, it is desirable that sufficient numbers of cations are injected into the ion trap to reach the maximum ion storage capacity within a few milliseconds and that these numbers are maintained throughout the ion/ion reaction period. The abundant low mass-to-charge ratio ions are ejected immediately prior to mass analysis

to avoid the deleterious effects of the positive ion electric field on mass analysis of the negative ions.

Cations of molecular oxygen have been used to demonstrate the concept of trapping by proxy. The most abundant ion formed by positive glow discharge of air is $O_2^{\bullet+}$ and it is expected to react with oligonucleotide anions by electron transfer. The preliminary study that demonstrated trapping by proxy suggested very little fragmentation associated with the use of $O_2^{\bullet+}$ as a charge reducing agent for mixed-base oligonucleotides [7]. However, anions derived from polydA oligomers showed evidence suggestive of fragmentation in ion/ion reactions with $O_2^{\bullet+}$. Fig. 1 shows the post-ion/ion reaction mass spectrum, adapted from the original study [7], of a 5′-pd$(A)_{40–60}$-3′ mixture using $O_2^{\bullet+}$ as the charge manipulation reagent (400 ms reaction time at a low-mass cutoff of 25). Ions corresponding to each of the 40- to 60-mer components are clearly present in the spectrum. However, relatively abundant signals corresponding to 20- to 39-mers, and even smaller oligomers, are evident. Doubly or triply charged 40- to 60-mers can contribute to some of the signals in the region below about *m/z* 12,150 but such products cannot account for most of the signal in this region. It was speculated that the 5′-pd$(A)_{20–39}$-3′ ions are series of fragment ions arising from the multiple electron-transfer reactions with $O_2^{\bullet+}$ experienced by the 5′-pd$(A)_{40–60}$-3′ ions.

To investigate further the ion/ion reaction behavior of the polydeoxyadenylate and other homopolymers, single component solutions of a variety of oligomers were sprayed and subjected to ion/ion reactions with $O_2^{\bullet+}$. The sample set included 5′-d$(A)_{20}$-3′, 5′-d$(C)_{20}$-3′, 5′-d$(T)_{20}$-3′, and a poly CG 20-mer, 5′-d$(CGGG)_5$-3′. The synthesis of 5′-d$(G)_{20}$-3′ is problematic. When more than four guanine residues are in a row, they tend to form a "guanine quartet" [46]. This lends high thermal stability to the structure and interferes with the synthesis of long polydG oligomers. Therefore, every fourth "G" was replaced with a "C" from the 3′-end to prevent the formation of the quartet. The $[M - 7H]^{7-}$ ion of each oligomer was isolated for exposure to the intense beam of $O_2^{\bullet+}$. Fig. 2 represents the post-ion/ion reaction spectra of the 5′-d$(A)_{20}$-3′, 5′-d$(C)_{20}$-3′, 5′-d$(T)_{20}$-3′, and 5′-d$(CGGG)_5$-3′ anions with $O_2^{\bullet+}$. The abundance scales are expanded by a factor of 16 relative to the full-scale abundance of the intact singly charged species, to highlight the low-abundance product ions.

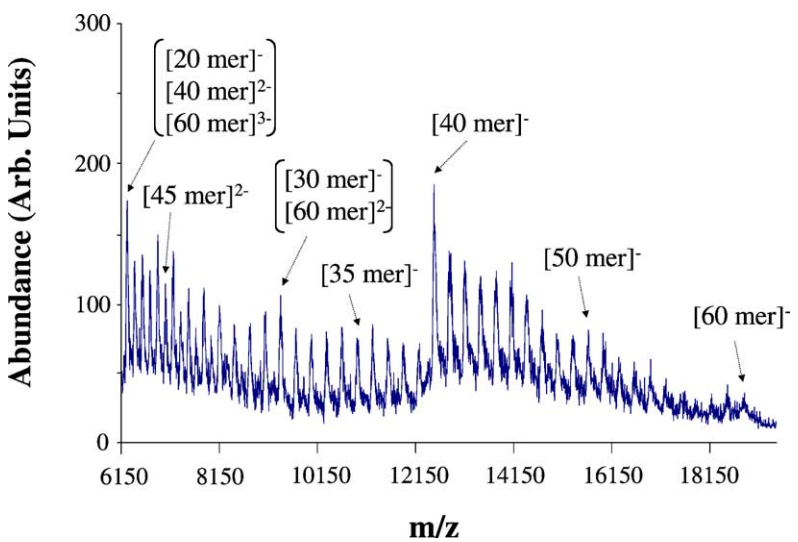

Fig. 1. Post-ion/ion reaction mass spectrum of the 5′-pd$(A)_{40–60}$-3′ mixture using $O_2^{\bullet+}$ as the charge manipulation reagent. $O_2^{\bullet+}$ ions were continuously admitted into the ion trap for 400 ms at an LMCO of 25.

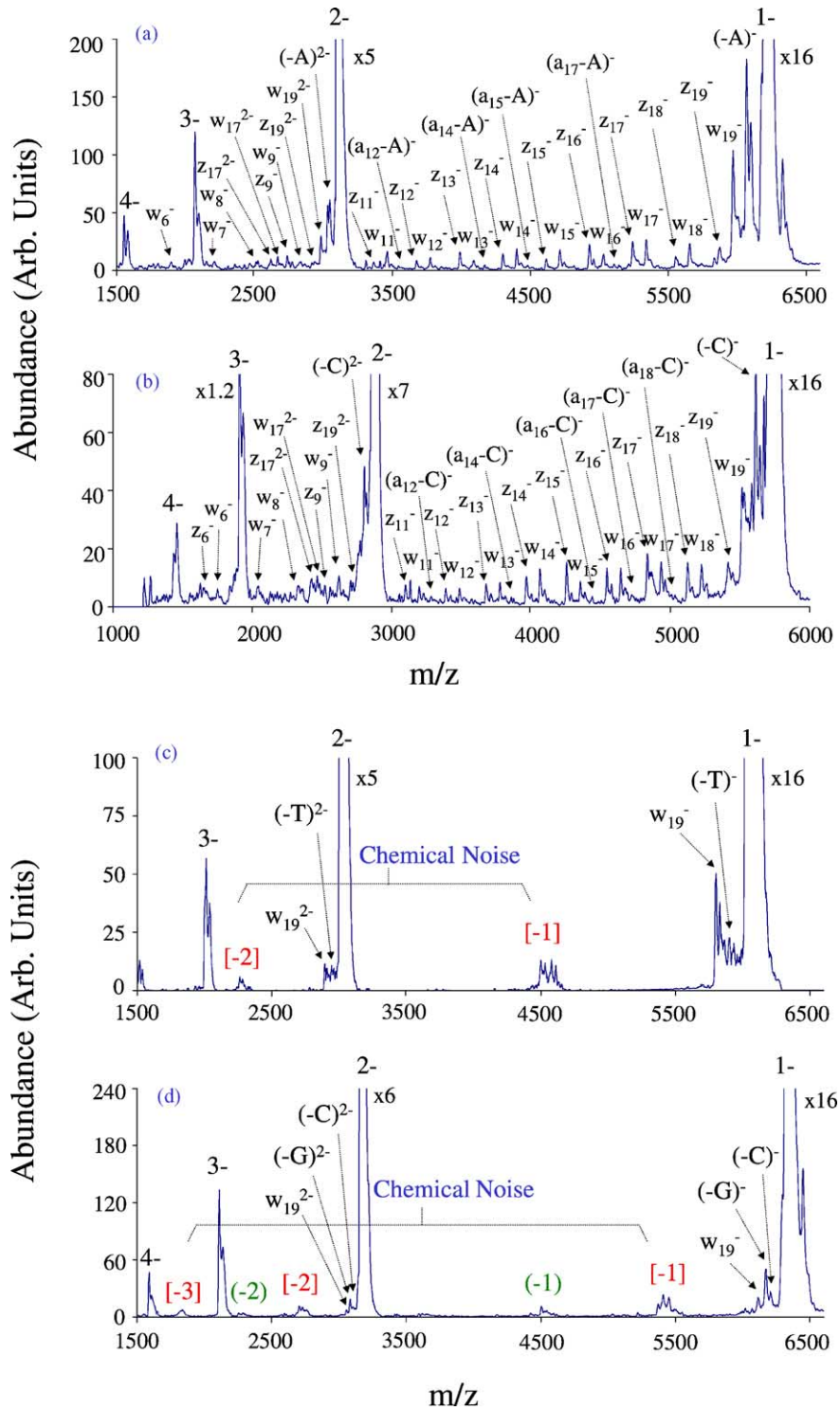

Fig. 2. Post-ion/ion reaction mass spectra of the $[M-7H]^{7-}$ ions of (a) 5′-d(A)$_{20}$-3′, (b) 5′-d(C)$_{20}$-3′, (c) 5′-d(T)$_{20}$-3′, and (d) 5′-d(CGGG)$_5$-3′ using $O_2^{\bullet+}$ as the charge reducing agent. The vertical scale was blown up by a factor of 16 to highlight the low-abundance product ions.

Charge states are driven mostly down to −1 and −2, although a small amount of intact triply and quadruply charged molecular ions are apparent. All the oligomers showed loss of a base and w_{19} ions as the major fragment ions after reacting with $O_2^{\bullet+}$. In addition, the homopolymers of polydA (Fig. 2a) and polydC (Fig. 2b) clearly showed formation of a series of w- (or d-) and z-type fragment ions extending down to at least the w_6 level, as well as some low-abundance (a_n-B_n) ions. The w- and d-type ions are not distinguishable based on mass-to-charge ratios alone because of the symmetry of the homopolymers. The 5′-d$(A)_{20}$-3′ results support the hypothesis that oligomer ions of $n \leq 39$ observed in Fig. 1 arise from fragmentation induced by electron transfer. Each polydA component undergoes multiple electron transfer reactions with $O_2^{\bullet+}$ and yields z_{n-x} and w_{n-x} fragments. A w_{n-1}-type ion from a 5′-d$(A)_n$-3′ anion is essentially the next smaller 5′-phosphorylated oligomer, 5′-pd$(A)_{n-1}$-3′. All w-type fragments contribute to the signals that nominally correspond to 5′-pd$(A)_n$-3′ ions in Fig. 1. The data of Fig. 2a suggest that z-type ions should also be present in Fig. 1. If so, they are likely to be obscured in the relatively noisy baseline.

In contrast with the results for polydA and polydC, no evidence for z-, w-, and a-B fragment ions is noted for the 5′-d$(T)_{20}$-3′ (Fig. 2c) and 5′-d$(CGGG)_5$-3′ anions (Fig. 2d). Low-abundance signals that arise from chemical noise are apparent in these spectra. They result from species selected in the ion isolation process that have different masses and charges than those of the parent ion of interest. These post-ion/ion reaction "chemical noise" peaks are indicated according to their reduced charge states. For example, peaks labeled [-1], [-2], [-3] in Fig. 2d arise from a -6 species of approximate mass 5412 Da. The peaks labeled (-1) and (-2) represent products from -5 species with masses of approximately 4510 Da.

The tendency for fragmentation as a result of electron transfer reactions with $O_2^{\bullet+}$ is greatest for the polydA and polydC homopolymer anions and least for the mixed-base CG oligomer and polydT anions. This tendency does not correlate strongly with the order of proton affinities of the nucleobases, which is G > A, C > T [47]. It is not surprising that the polydT anion appears to be more stable than the polydA and polydC anions. PolydT ions have been noted to be particularly stable in previous oligonucleotide fragmentation studies [48–51], and this stability has been attributed to the relatively low proton affinity of the thymine nucleobase. However, the apparent stability of the 5′-d$(CGGG)_5$-3′ anions suggests that nucleobase proton affinity alone cannot account for the observed tendency for fragmentation as a result of electron transfer. In this case, interactions that lend thermal stability to polydG oligomers may also contribute to the apparent enhanced stability of the G-rich GC oligomer studied here, relative to the anions of polydC and polydA. In any case, further systematic studies are warranted before firm conclusions can be drawn regarding the sequence dependence associated with fragmentation arising from electron transfer reactions of oligonucleotide anions in the gas phase.

The product ions resulting from electron transfer of 5′-d$(A)_{20}$-3′ and 5′-d$(C)_{20}$-3′ to $O_2^{\bullet+}$ are also worthy of note. The most prominent fragmentation channel of oligonucleotide anions under quadrupole ion trap collisional activation conditions is the loss of a nucleobase followed by cleavage of the 3′ C–O bond of the sugar from which the base was lost to yield the complementary w-type and (a-B)-type ions [52–55]. Both w- and (a-B)-ions are identified in the post-ion/ion reaction spectra of the 5′-d$(A)_{20}$-3′ and 5′-d$(C)_{20}$-3′ anions in reactions with $O_2^{\bullet+}$. These product ions can arise from the same unimolecular processes as those resulting from collisional activation of even-electron anions. While distinct mechanisms cannot be precluded for radical containing ions, no evidence is apparent that requires invoking new mechanisms. The most striking observation is the prominent appearance of a series of z-ions, which are rarely observed via ion trap collisional activation of the corresponding even-electron ions. Differences observed between the ion trap collisional activation of even-electron anions and electron transfer induced dissociation of odd-electron anions could arise from differences in the activation conditions (i.e., internal

energies and time-frames) or different unimolecular dissociation chemistries.

To shed light on the origin of the z-type ions we conducted experiments that allow for similar activation conditions to be applied to even- and odd-electron species of the same charge state. Data for a mixed-base 12-mer, 5′-d(CTTAGCGCTAAG)-3′, illustrates our findings. Fig. 3 shows the MS/MS spectrum of $[M - 4H]^{4-}$ (Fig. 3a) of the 12-mer formed directly via nano-ESI and the MS/MS spectrum of the $[M - 6H]^{\bullet\bullet 4-}$ ion (Fig. 3b) formed by double electron transfer to $O_2^{\bullet +}$ from the $[M - 6H]^{6-}$ ion. Any differences in the internal energies of the parent ions prior to ion activation tend to be removed by ion/helium collisions in the ion trap. Very similar activation conditions for the two 4- species were employed (i.e., the ions were subjected to identical activation amplitudes and the amplitude of the main trapping voltage was adjusted so that the two ions had the same resonance excitation frequency). Therefore, the results in Fig. 3 are expected to reflect dissociation behavior under very similar time-scales and ion internal energies. Perhaps the most significant observation in the comparison of the spectra of Fig. 3 is their high degree of similarity. Both 4- parent ions fragment via a common set of major dissociation channels showing loss of neutral bases and a series of w and (a-B) complementary ions. All of the backbone cleavages were observed for the $[M - 4H]^{4-}$ ion except at the 3′-side of the thymidine residues. The ions of w_6^{2-} and $(a_{10}\text{-A})^{3-}$ are labeled together due to the fact that the *m*/*z* values of these ions could not be resolved.

The similarity of the dissociation behaviors of the two parent anions represented in Fig. 3 suggests that

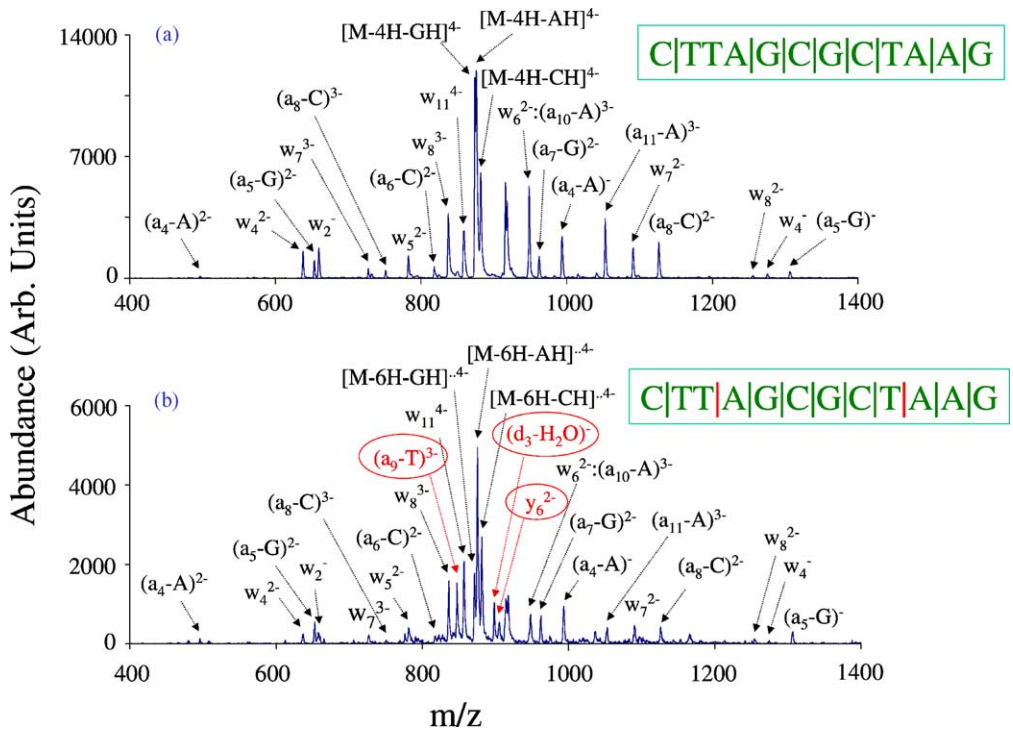

Fig. 3. (a) CID MS/MS spectrum of $[M - 4H]^{4-}$ of 5′-d(CTTAGCGCTAAG)-3′ derived directly from nano-ESI. Activation was performed at a resonance excitation frequency of 40,130 Hz at 230 mV for 200 ms using an LMCO of 100.2. (b) CID MS/MS spectrum of the $[M - 6H]^{\bullet\bullet 4-}$ ion resulting from the reaction of $[M - 6H]^{6-}$ of the 12-mer with $O_2^{\bullet +}$ for 45 ms at an LMCO of 25. Activation conditions were the same as in (a) except that an LMCO of 100 was used.

the presence of radical sites does not introduce major new facile fragmentation channels. A few subtle differences are noted, however (see below). Furthermore, the similarity between the fragmentation behaviors of the parent ions formed by direct nano-ES and double electron transfer is consistent with the independence of the radical sites in the $[M - 6H]^{\bullet\bullet 4-}$ ion. If the radical sites in this species, which are expected initially to be found at phosphodiester linkages, were to combine to form a stable ring within the oligonucleotide, the MS/MS spectrum might be expected to differ more significantly from the MS/MS spectrum of the $[M - 4H]^{4-}$ ion. However, definitive conclusions cannot be drawn because data showing the dissociation behavior of an ion known to contain bonding between phosphodiester linkages are not in hand.

A few product ions of relatively low abundance are formed in the dissociation of the radical species that are absent in the MS/MS spectrum of the $[M - 4H]^{4-}$ ion. For example, the $[M-6H]^{\bullet\bullet 4-}$ ion gives rise to product ions assigned as $(a_9\text{-}T)^{3-}$, $(d_3\text{-}H_2O)^{-}$, and $y_6{}^{2-}$. (Note that the confidence with which these assignments are made is less than those associated with Fig. 2 because the product ions were not reduced to singly charged ions. The use of $O_2{}^{\bullet +}$ to manipulate product ion charge states would introduce ambiguity in the interpretation of the product ion spectra.) There are also differences in the relative abundances of some of the product ions held in common for the two parent ions but they are relatively small and it is not clear how significant they are.

The fact that the parent ions of Fig. 3 show very similar fragmentation behavior under a common set of ion activation conditions, and that z-type ions are not noted in the data of Fig. 3, suggests that the z-type ions that appear in Fig. 2a and b result from the time-scales and energies associated with the ion/ion reactions. Ion trap collisional activation is a continuous and relatively slow process that typically gives rise to fragmentation reactions in the 10–100 s^{-1} rate range [56,57]. In the case of ion/ion reactions, energy partitioned into the charge transfer products is expected to occur relatively rapidly (e.g., in conjunction with the charge transfer event). Ion activation associated with an ion/ion reaction is, therefore, expected to be relatively rapid compared with ion trap collisional activation. The likelihood for fragmentation to occur in an ion trap as a result of an ion/ion reaction is related to the partitioning of reaction exothermicity and relative translation of the ions into internal modes of the charge transfer products, the dissociation kinetics of the ions, and the cooling rates associated with ion/bath gas collisions. A simulation of the cooling rates associated with polypeptide ions under typical quadrupole ion trap storage conditions suggested that dissociation rates in excess of roughly 10^4 s^{-1} following a rapid input of energy is required to observe a significant degree of fragmentation [58]. All cation/anion neutralization reactions are expected to be highly exothermic [30], although the relative partitioning of this energy between internal and translational modes has not been established. A plausible scenario for the observation of the z-type ions from the polydA and polydC anions is that they arise from relatively fast dissociation reactions driven by the ion/ion reaction exothermicity. Evidence for fast cleavages giving rise to z-type product ions from oligonucleotide ions has been reported. Chan et al. [59] studied prompt and metastable dissociations of adenine–thymine binary-base oligonucleotides in positive ion MALDI-UV-TOF using 2-aminobenzoic acid/ammonium fluoride as the matrix. Post-source decay yielded predominantly $(a\text{-}B)^{+}$ and w^{+} complementary ion series whereas in source dissociation generated $[z\text{-}AH]^{+}$, w^{+}, d^{+}, and other product ions.

3.2. Proton transfer

3.2.1. Protonated isobutylene ($C_4H_9{}^{+}$)

The fragmentation associated with electron transfer reactions of polydA and polydC anions indicates that the use of $O_2{}^{\bullet +}$ as a charge state manipulation reagent in oligonucleotide mixture analysis could be problematic. The trapping by proxy approach is not restricted to oxygen cations, however, provided that the singly charged ions can be injected into the ion trap at relatively high abundances. We examined the use of isobutane as the discharge support gas instead of air in light of the fact that it is a commonly used

reagent in chemical ionization [60]. The $C_4H_9^+$ ions can be formed in sufficiently high abundance to effect the trapping by proxy approach. In this case, proton transfer is expected to be the mechanism for charge reduction, which is the preferred mechanism for producing a homogeneous singly charged ion population from each parent ion charge state [29]. The reactions of the same set of homopolymers with $C_4H_9^+$ were conducted. The post-ion/ion reaction spectra of $[M-4H]^{4-}$ of 5′-d$(A)_{20}$-3′ and 5′-d$(T)_{20}$-3′ are shown in Fig. 4. These two homopolymers were selected for demonstration because they represent both the reactive and non-reactive homopolymers. The singly deprotonated molecules are scaled up by a factor of 12. Significantly less fragmentation is induced compared to reactions with $O_2^{\bullet+}$. Only a small degree of base loss and w_{19}^- ion formation is noted with no obvious adduct formation or other decomposition. Chemical noise peaks are apparent in the spectra reflecting upon the purity of the oligonucleotide samples. The reaction of the 5′-pd$(A)_{40–60}$-3′ mixture with $C_4H_9^+$ also showed little fragmentation, and each mixture component was clearly distinguishable (see Fig. 7 in [7]). These results suggest that only a minimal degree of fragmentation is associated with proton transfer reactions from $C_4H_9^+$ to the oligonucleotide anions.

The largest oligonucleotide we have examined thus far with the “trapping by proxy” approach is a 5S ribosomal RNA from *E. coli* with 120 bases (MW 38.9 kDa). Fig. 5 compares the pre- and post-ion/ion reaction mass spectra derived from negative nano-ES of an 8 μM solution of 5S rRNA using $C_4H_9^+$ as the charge reducing agent. The post-ion/ion spectrum was acquired by injecting $C_4H_9^+$ ions for 300 ms at an LMCO of 35. Intact singly, doubly, and triply charged 5S rRNA ions are clearly apparent (Fig. 5b). Peaks are observed at masses lower than that of the intact 5S rRNA in Fig. 5b, which are likely due to the impurities present in the sample. The continuous injection of $C_4H_9^+$ ions at an LMCO of 35 permits

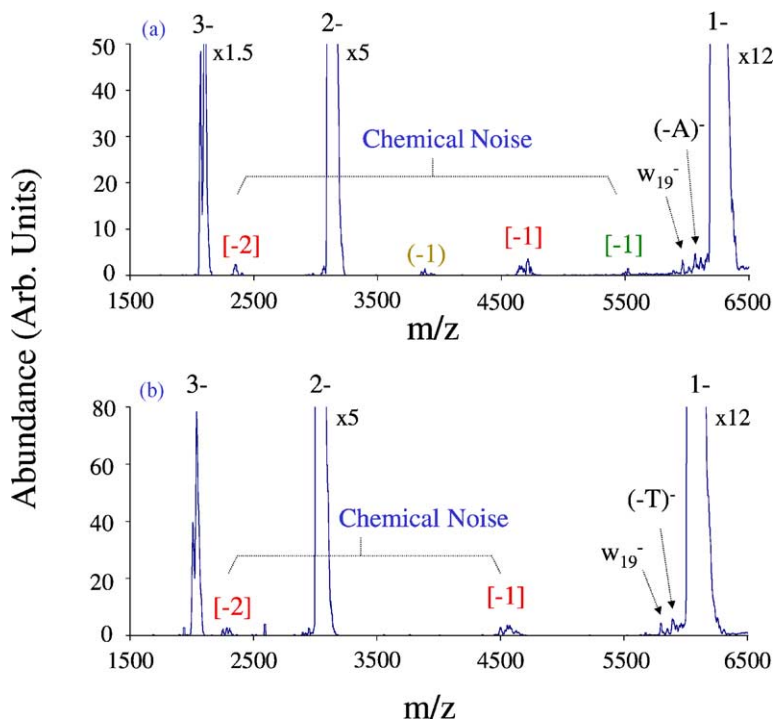

Fig. 4. Post-ion/ion reaction mass spectra of the $[M-4H]^{4-}$ ions of (a) 5′-d$(A)_{20}$-3′, (b) 5′-d$(T)_{20}$-3′ using $C_4H_9^+$ as the charge manipulation reagent.

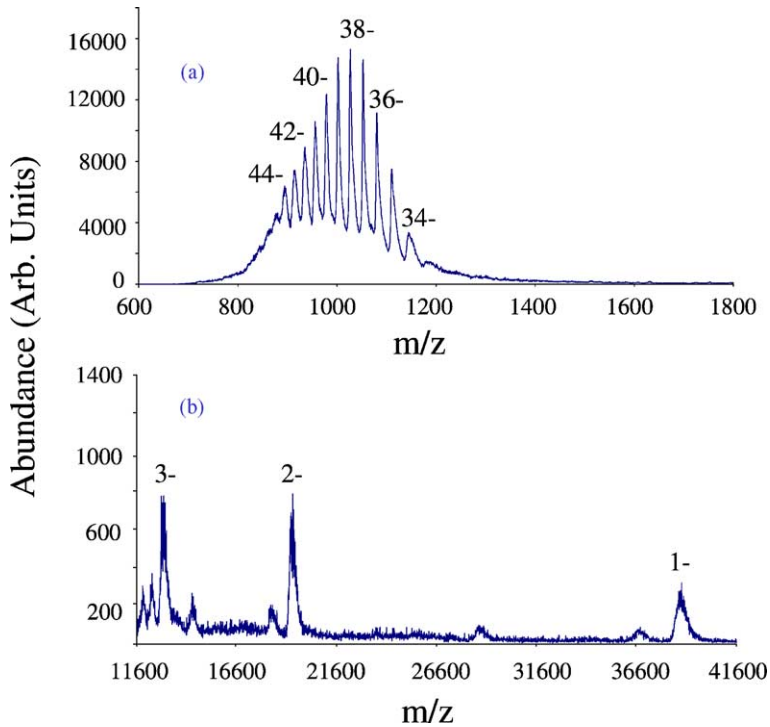

Fig. 5. (a) Pre- and (b) post-ion/ion reaction mass spectra of 5S ribosomal RNA from *E. coli* with $C_4H_9^+$ ions continuously injected for 300 ms at an LMCO of 35.

the observation of singly charged ions as high in *m*/*z* as 39,000.

3.2.2. Benzoquinoline

Another solution to the problem of mutual ion storage over a broad mass-to-charge ratio range is to form high-mass cations via another ionization method, such as ESI. Trapping by proxy may not be practical for ion sources less bright than glow discharge ionization but is not necessary if high mass-to-charge ratio singly charged ions can be formed. The recent development of the "dueling electrospray" ion trap in our laboratory [39] enables this tactic to be evaluated. This instrument allows for sequential injections of ions of opposite polarities, both formed by nano-ESI, through an ion trap end-cap electrode. Protonated pyridine, formed by ion trap chemical ionization, has been reported to react with multiply charged 5′-d(AAAA)-3′ anions exclusively by proton transfer with very little fragmentation or adduct formation [23]. We, therefore, looked into the ESI of pyridine and related compounds as candidates for oligonucleotide charge manipulation reagents. Protonated pyridine, quinoline, isoquinoline, and BQ, derived from nano-ESI of 1% acetic acid solution with a concentration of 1 mg/mL, respectively, all yielded protonated molecules of relatively high abundance. Each was observed to undergo proton transfer reactions with multiply charged oligonucleotide ions. BQ (MW 179 Da) has the highest mass among the four molecules tested and should, therefore, be most useful for reduction of the charge states of high-mass oligonucleotides. Furthermore, it has been used to react with multiply charged protein anions to reduce charge without leading to either fragmentation or attachment [15,39]. The reactions of the $[M - 7H]^{7-}$ of the 20-mers discussed above with protonated BQ (BQH^+) were carried out and the results are summarized in Fig. 6. Singly, doubly,

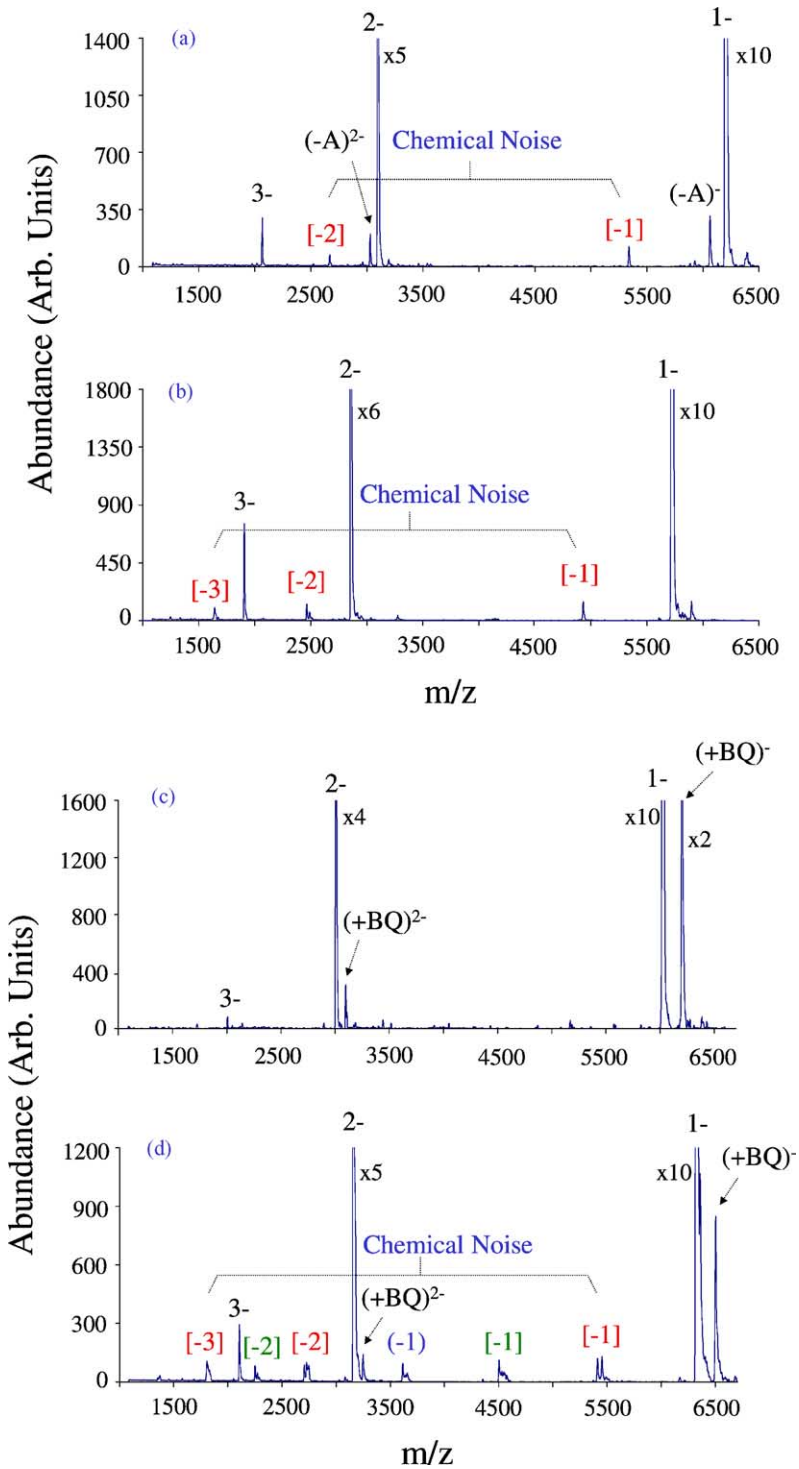

Fig. 6. Post-ion/ion reaction mass spectra of the $[M-7H]^{7-}$ ions of (a) 5′-d$(A)_{20}$-3′, (b) 5′-d$(C)_{20}$-3′, (c) 5′-d$(T)_{20}$-3′, and (d) 5′-d$(CGGG)_5$-3′ with the use of protonated BQ (BQH^+) as the charge reducing agent.

and triply charged oligomers are observed with very little fragmentation. Only a very small amount of adenine loss from 5′-d(A)$_{20}$-3′ (Fig. 6a) is observed, whereas essentially no fragment ions are seen for the 5′-d(C)$_{20}$-3′, 5′-d(T)$_{20}$-3′, and 5′-d(CGGG)$_5$-3′ ions (Fig. 6b, c, and d). (Fragmentation of the polydA anion during the course of ion isolation cannot be precluded from giving rise to the small degree of base loss observed.) Interestingly, a small degree of attachment of BQ to each of the 20-mers is noted with the proportion of attachment in the singly charged species being noticeably greatest for the 5′-d(T)$_{20}$-3′ (Fig. 6c) and 5′-d(CGGG)$_5$-3′ (Fig. 6d) species. Various reaction times were applied and consistently showed that proton transfer was the predominant process for all charge states and that adduct formation tended to occur late in the sequence of charge reduction. That is, adduct ions were observed only at lowest charge states (−1 and −2) but not at −3 or higher charge states. Collisional activation of the $[M + BQ - H]^-$ ion of 5′-d(T)$_{20}$-3′ yielded the $[M - H]^-$ ion and the neutral BQ as products without other fragments (data not shown) indicating that the adduct is loosely bound. Protonated BQ has been used extensively to react with a large number of mixed-base oligonucleotide anions in our laboratory (unpublished data). Essentially exclusive proton transfer has been consistently observed (i.e., adduct formation and fragmentation have been largely absent). The results for the polydT and 5′-d(CGGG)$_5$-3′ ions are somewhat exceptional in the extent of adduct formation observed. Nevertheless, this result indicates that the nucleobases can play a role in the binding of BQ to the oligomer and that adduction may not simply be an interaction between the basic nitrogen of BQ and the acidic proton of a phosphodiester linkage.

The relative tendencies for fragmentation associated with ion/ion reactions in the ion trap are most apparent when the oligonucleotide is relatively small. This is expected for ions that show statistical fragmentation behavior whereby, for a given internal energy transfer upon ion/ion reaction, ion lifetime is inversely related to the number of degrees of freedom. Collisional cooling is less likely to inhibit fragmentation resulting from ion/ion reactions when the fragmentation rate is comparable to or faster than the cooling rate. Fig. 7 compares the ion/ion reaction spectra arising from interactions of the $[M - 3H]^{3-}$ ion derived from 5′-d(AAAA)-3′ with $O_2^{\bullet+}$, $C_4H_9^+$, and BQH^+ ions. The reaction with $O_2^{\bullet+}$ (Fig. 7a) leads to extensive fragmentation with the observation of essentially all of the possible w- and (a-A)-type fragments as well as the y_3 ion. A singly charged ion of the intact oligonucleotide is not observed despite the use of an ion/ion reaction time sufficiently long for it to be observed in the absence of fragmentation. This is similar to the reaction spectra of the same oligonucleotide anion with $Xe^{\bullet+}$ [24,31], whose recombination energy (12.1 eV, [61]) is close to that of $O_2^{\bullet+}$. The fragment ion indicated with a # sign in Fig. 7a corresponds to a loss of a terminal base and part of a terminal sugar [24]. The reaction with $C_4H_9^+$ (Fig. 7b) results in abundant proton transfer products, doubly and singly charged oligonucleotide anions, although a small number of w_2, w_3, and (a_2-A_2) fragment ions are apparent. The reaction with BQH^+ (Fig. 7c) yields exclusive proton transfer products with little, if any, fragmentation or adduct formation.

The ion/ion reaction exothermicity provides the major source of energy to drive fragmentation. Reaction exothermicity for the electron transfer reaction (see process 1) is given as:

$$\Delta H_{rxn} = EA([M - nH]^{\bullet(n-1)-}) - RE(O_2^{\bullet+}) \quad (4)$$

where $EA([M - nH]^{\bullet(n-1)-})$ is the electron affinity of the product anion and $RE(O_2^{\bullet+})$ is the recombination energy of $O_2^{\bullet+}$. The $RE(O_2^{\bullet+})$ involving ground state ionization and ground state neutral is the adiabatic ionization potential (IP) of O_2 (12.1 eV, [62]). The $EA([M - nH]^{\bullet(n-1)-})$ is estimated to be small in magnitude (<1 eV) [24]. The enthalpy of this reaction is, therefore, expected to be approximately −11 eV.

The enthalpies of proton transfer reactions of oligonucleotide anions with $C_4H_9^+$ and protonated BQ are determined by the differences in the proton affinities (PA), i.e.,

$$\Delta H_{rxn} = PA(C_4H_8) - PA([M - nH]^{n-}) \quad (5)$$

$$\Delta H_{rxn} = PA(BQ) - PA([M - nH]^{n-}) \quad (6)$$

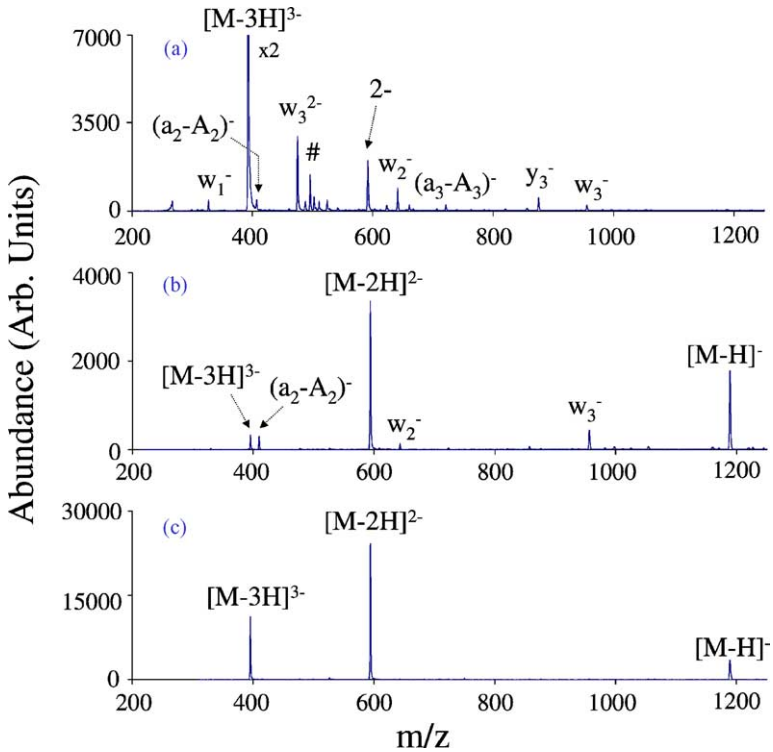

Fig. 7. Post-ion/ion reaction mass spectra of the $[M - 3H]^{3-}$ ion of 5′-d(AAAA)-3′ with (a) $O_2^{\bullet+}$, (b) $C_4H_9^+$, (c) BQH^+ as the charge manipulation reagent, respectively.

The proton affinities of BQ and $[M - nH]^{n-}$ ions of the oligonucleotides have not been measured. Semi-empirical calculations were, therefore, performed to obtain estimates of these values. The $(CH_3O)_2OPO^-$ anion was used as a model of deprotonated nucleotides without considering the effect of multiple deprotonation and the nucleobases. Calculations gave a PA of 329.8 kcal/mol, equivalent to 14.3 eV, for the $(CH_3O)_2OPO^-$ ion and a PA of 229.6 kcal/mol (10.0 eV) for BQ. Therefore, the enthalpy of reaction with BQH^+ is roughly −4.3 eV, without considering the effect of multiple deprotonation of the oligonucleotide. The proton affinity of C_4H_8 is 8.3 eV [63]. Therefore, the heat of reaction with $C_4H_9^+$ is estimated to be at least −6 eV. Multiple charging of the oligonucleotide increases the exothermicities of all of the reactions. Therefore, the order of the reaction exothermicities associated with the various reagents ions is $O_2^{\bullet+} > C_4H_9^+ > BQH^+$ which correlates with the degree of fragmentation observed with the 5′-d(AAAA)-3′ ions observed in Fig. 7.

The lesser degree of fragmentation associated with ion/ion reactions with BQH^+ and $C_4H_9^+$ relative to $O_2^{\bullet+}$ is likely to be more related to differences in ion/ion reaction energetics than to differences in the stabilities of radical-containing ions formed via electron transfer vs. the even-electron products formed via proton transfer reactions. Based on studies like those described in association with Fig. 3, it is apparent that there are not significant differences in the kinetic stabilities of the proton transfer vs. the electron transfer products.

3.3. Ion parking of oligonucleotide anions

Use of BQ has an added advantage of being able to employ the “ion parking” technique [45]. By accelerating one of the reactant ions at mass-to-charge-

dependent frequencies of motion, it is possible to diminish selectively the rates of ion/ion reactions in a quadrupole ion trap. The application of a resonance excitation voltage tuned to inhibit the ion/ion reaction rate of a specific range of ion mass-to-charge ratios is termed ion parking [45]. The ion parking technique has been demonstrated with positive multiply charged protein ions using anions derived from glow discharge ionization of perfluoro-1,3-dimethylcyclohexane (PDCH) as the charge manipulation agent [14,43,45]. It facilitates a number of useful analytical applications, including the "concentration" of ions initially dispersed over a distribution of charge states into a selected charge state [14,43,45], selection of a particular ion from a set of ions derived from a complex protein mixture [43,45], and charge-state purification of protein ions in the gas phase for subsequent ion activation by use of a double ion isolation experiment involving two ion parking and two isolation steps [43].

The ion parking approach is effective only when the electric field associated with the presence of the oppositely charged ions is sufficiently small that it does not seriously affect the resonance excitation process [45]. This requirement is not met by the conditions under which the trapping by proxy approach works. Therefore, $O_2^{\bullet+}$ and $C_4H_9^+$ ions, when used as proxies for trapping high mass-to-charge ratio anions, cannot be used for ion parking. Trapping by proxy, however, is not necessary for high-*m*/*z* cations such as protonated BQ. Hence, protonated BQ was examined for ion parking experiments. Ions derived from nano-ESI of a mixed-base 50-mer are used as an illustration shown in Fig. 8. Fig. 8a shows the isolated $[M-15H]^{15-}$ ion of the 50-mer. Fig. 8b represents the spectrum after cations of BQ were admitted into the ion trap for 60 ms and a mutual anion/cation storage time of 300 ms was used prior to cation ejection and subsequent mass analysis (i.e., the normal post-ion/ion reaction mass

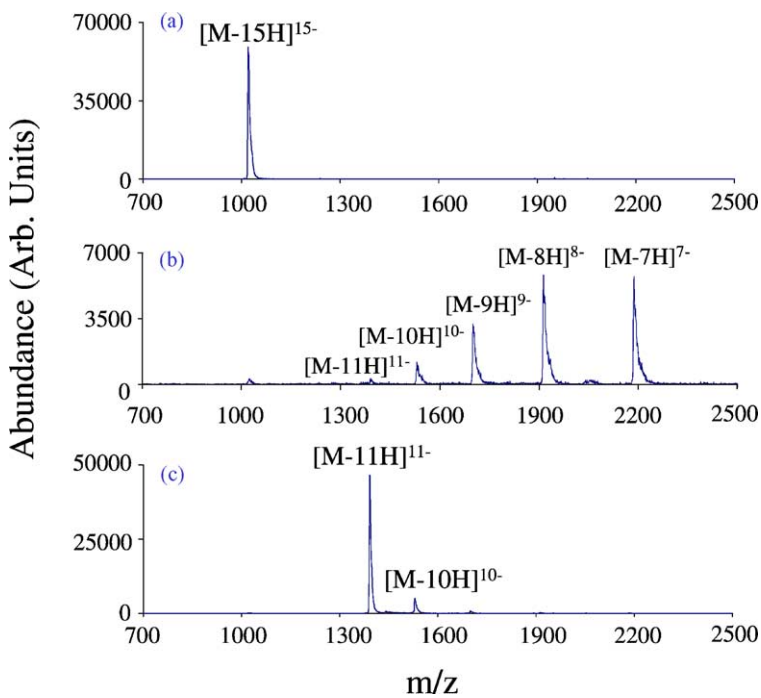

Fig. 8. (a) Mass spectrum of isolated $[M-15H]^{15-}$ of a 50-mer. (b) Post-ion/ion mass spectrum of the 50-mer using BQH^+ ions for charge manipulation. The cation injection and mutual anion/cation storage periods employed were 60 and 300 ms, respectively. (c) Mass spectrum acquired in the ion parking mode using the same conditions as described in (b), with the addition of a 1.3 V_{p-p} resonance excitation voltage at 37,400 Hz during the mutual ion storage period.

spectrum). A range of low charge states, −11 to −7, was yielded. Lower charge states of the 50-mer were also formed but they fell outside of the mass-to-charge range over which data were collected. Fig. 8c shows the results of an experiment with ion/ion reaction conditions identical to those used in Fig. 8b except that a single dipolar frequency of 37.4 kHz and 1.3 V_{p-p} was applied to the end-cap electrodes during the ion/ion mutual storage period. It is apparent that the extent of proton transfer in Fig. 8c has been significantly reduced relative to Fig. 8b. By accelerating the −11 charge state ions as they are formed, the ion/ion reaction rate for this charge state is greatly diminished, thereby allowing the signal to be "concentrated" in this charge state, resulting in an abundance close to that of the initial −15 charge state. A small number of lower charge states are also observed indicating that the ion parking conditions used in this experiment did not lead to a complete inhibition of the reaction rate of the −11 charge state. It is demonstrated here that the ion parking process can be readily effected with multiply charged anions provided the necessary conditions for ion parking can be established.

3.4. Proton transfer vs. cation attachment

It is of interest to identify higher mass-to-charge ratio positive ions than that of protonated BQ for the charge state manipulation of oligonucleotides of mass greater than about 50 kDa. For this reason, we examined the charge state manipulation of oligonucleotide anions with small singly charged peptides. To illustrate, we present results for LE, a five-amino acid peptide (YGGFL, MW 555.6 Da) without any acidic or basic residues. Nano-ESI yields the singly protonated molecule, $[LE + H]^+$, from a 1% acetic acid aqueous solution. The $[M - 4H]^{4-}$ anion of the 12-mer was subjected to reaction with $[LE+H]^+$. The product ion spectrum is shown in Fig. 9a. An anion formed by the attachment of one LE to the 12-mer, $[M + LE - 3H]^{3-}$, is the most abundant first generation product along with some single-proton transfer

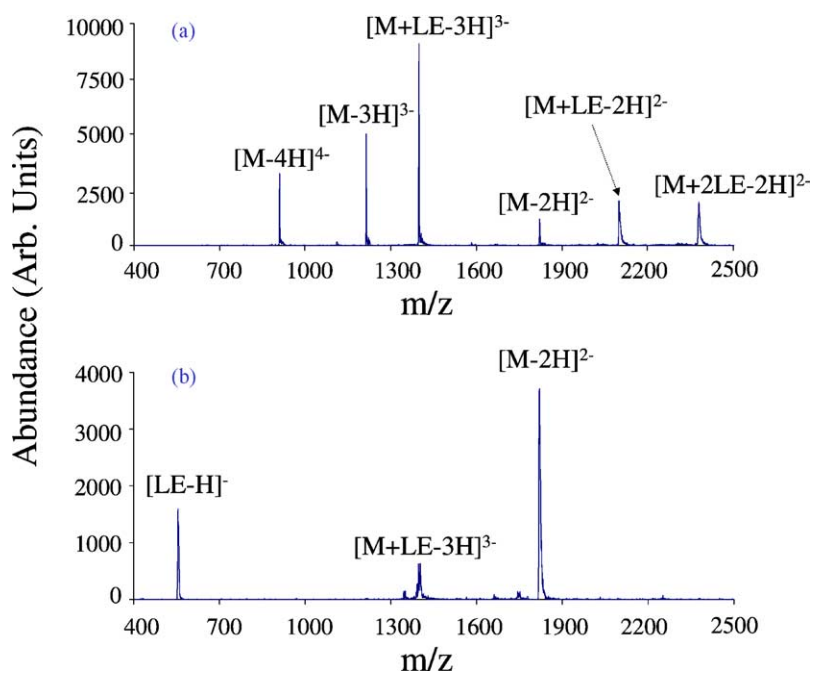

Fig. 9. (a) Post-ion/ion reaction mass spectrum of $[M - 4H]^{4-}$ of the 12-mer with protonated LE. (b) CID MS/MS spectrum of the adduct ion, $[M + LE - 3H]^{3-}$, isolated from (a).

product, $[M - 3H]^{3-}$ of the 12-mer. Sequential attachment ($[M + 2LE - 2H]^{2-}$) and proton transfer ($[M-2H]^{2-}$) products are also evident in the spectrum along with $[M + LE - 2H]^{2-}$, which can be formed from $[M - 3H]^{3-}$ and $[M + LE - 3H]^{3-}$ via LE attachment and proton transfer, respectively. Although it has been known that DNA–protein complexes can be generated readily in solution via non-covalent interactions [64,65], this is an example of the formation of an oligonucleotide–peptide complex exclusively in vacuo. However, LE is not expected to show specific association with the 12-mer in solution. Therefore, the adducts reflected in Fig. 9a are likely to arise from non-specific binding.

It is important to understand the factors that affect proton transfer vs. adduct formation in developing applications of ion/ion reactions. It has been noted previously in the reactions of multiply charged proteins with singly charged anions that the identity of the anion plays a major role in the tendency for adduct formation vs. proton transfer [66]. For the adducts observed to date with positively charged proteins, ion trap collisional activation of the adduct-containing ion results in the facile loss of the neutral adduct. In analogy, adducts of BQ with the 20-mers described above fragment by loss of neutral BQ upon ion trap collisional activation. This behavior is consistent with the picture that both proton transfer and adduct products from ion/ion reactions are formed from a single collision complex. The adducts are those complexes that are stable enough to survive the capture and ion storage conditions in the ion trap. This fraction increases with the strength of the interaction between the reactants. Stronger interactions lead to longer collision complex lifetimes thereby increasing the likelihood for collisional stabilization. However, recent results from the study of multiply charged anions in reactions with multiply charged cations have suggested the possibility for competing ion/ion reaction mechanisms such that some ion/ion reaction products might not be formed via a long-lived intimate collision complex [67]. For example, in some reactant ion trajectories, the transfer of a proton or electron might occur at a distance and relative velocity that avoids the formation of a long-lived collision complex [68]. This possibility should also exist for the reactions of multiply charged anions with singly charged cations. When the $[M + LE - 3H]^{3-}$ ion was subjected to ion trap collisional activation, the complex fragmented almost exclusively via the charge separation reaction to give the complementary $[M - 2H]^{2-}$ and $[LE - H]^{-}$ ions (see Fig. 9b). Interestingly, no $[M - 3H]^{3-}$ ions were formed. This suggests that either the $[M-3H]^{3-}$ ions observed in Fig. 9a were formed without going through a long-lived activated $[M+LE-3H]^{3-*}$ complex or that the energies and time-frames associated with decomposition of the complex under ion/ion reaction conditions can lead to the loss of neutral LE to give the $[M-3H]^{3-}$ ion. The latter scenario implies a significant difference in the entropies associated with breakup of the complex via loss of neural LE vs. loss of $[LE - H]^{-}$.

While the extent to which proton transfer occurs without the formation of a long-lived complex remains an open issue, it is clear that a significant fraction of ion/ion reactions proceed through a long-lived complex. This conclusion follows from the extensive clustering that is observed with particular types of ions. The extent of adduct formation has been correlated with binding strength of the adducts. It is, therefore, of interest to characterize the nature of binding associated with the attachment of peptide cations to oligonucleotide anions. To this end, we modified the C-terminal of LE via methyl esterification and the N-terminal via N-acetylation. In so doing, the most acidic and most basic portions of the molecule are affected. The post-ion/ion reaction spectrum of $[M - 4H]^{4-}$ of the 12-mer with the singly charged methyl ester of LE (LEE) is shown in Fig. 10a. Both adduct ions and proton transfer products are obtained, as observed with unmodified LE. The N-acetylated protonated LE gives the same result (data not shown). It appears that neither the C-terminus nor the N-terminus alone plays an essential role in the attachment of a peptide to an oligonucleotide anion. Apparently, the peptide, with its numerous polar groups along the peptide chain, can engage in multiple non-covalent interactions that can stabilize

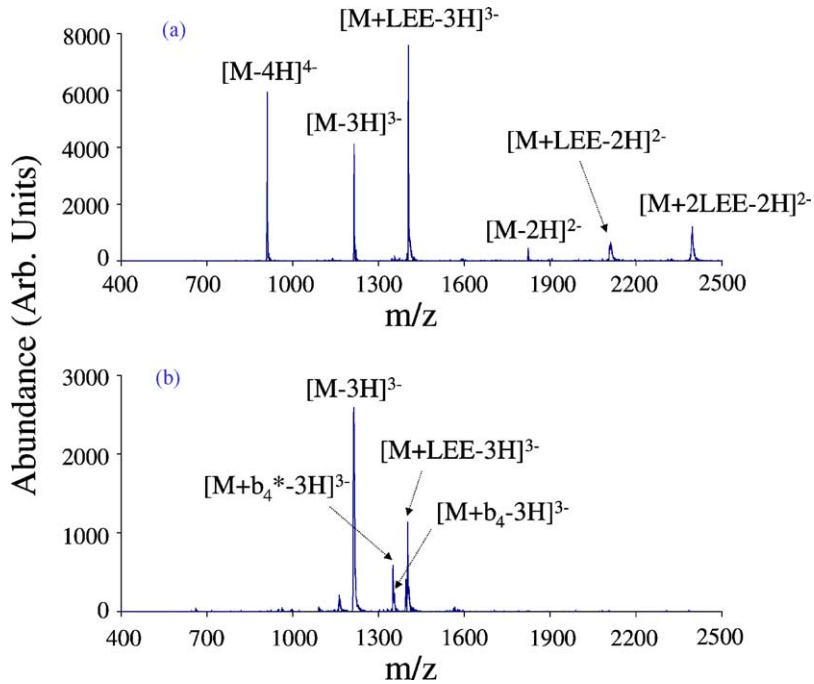

Fig. 10. (a) Post-ion/ion reaction mass spectrum of $[M-4H]^{4-}$ of the 12-mer with protonated methyl ester of LE (LEE). (b) CID MS/MS spectrum of the adduct ion, $[M+LEE-3H]^{3-}$, isolated from (a).

a complex with the oligonucleotide anion. This implies that peptide ions are not well-suited, regardless of amino acid composition, to manipulation of oligonucleotide charge states due to significant adduct formation.

The complex of $[M+LEE-3H]^{3-}$ was isolated and its ion trap CID MS/MS spectrum is shown in Fig. 10b. The $[M-3H]^{3-}$ anion appears as the dominant product and results from the loss of neutral LEE. Backbone cleavages of the LE ester without breaking the non-covalent bonds are also evident, indicated as $[M+b_4^*-3H]^{3-}$ and $[M+b_4-3H]^{3-}$ ions, where b_4^* is the loss of NH_3 from b_4 of the LE methyl ester. The complex ion $[M+LEE-3H]^{3-}$ does not produce the doubly charged DNA anion, $[M-2H]^{2-}$, presumably because the elimination of the C-terminal carboxy group by methyl esterification reduces the acidity of the peptide sufficiently to disfavor the charge separation channel, in contrast with the result for unmodified LE. Nevertheless, despite the absence of the C-terminal carboxy group, cleavage of the peptide backbone competes with the loss of LEE. This result provides further evidence for a relatively strong interaction, probably due to multiple weak interactions, between the peptide and oligonucleotide ions.

4. Conclusions

Experiments related here have provided new insights into ion/ion reactions involving multiply charged oligonucleotide anions. Electron transfer reactions involving $O_2^{\bullet+}$ give rise to fragmentation of a small but easily observed fraction of anions of polydA and polydC oligonucleotides. A lesser degree of fragmentation was noted for polydT anions as well as anions derived from 5′-d$(CGGG)_5$-3′. The loss of a nucleobase was noted for all anions and w- and (a-B)-type ions from throughout the oligomer were noted for polydA and polydC anions. Of particular

note was the formation of z-type fragment ions. The formation of these ions appears to be related to the short time-frames associated with ion/ion reaction fragmentation. A small degree of fragmentation of oligonucleotide anions can also result from proton transfer ion/ion reactions, as noted for reactions with $C_4H_9^+$. The degree of fragmentation observed appears to be directly related to the exothermicity of the ion/ion reaction. The kinetic stabilities of proton transfer and electron transfer products do not appear to be significantly different. Furthermore, both types of products fragment very similarly under ion trap collisional activation conditions. This observation indicates that the introduction of radical sites via ion/ion electron transfer reactions does not lead to major new fragmentation channels relative to those for the even-electron ions of the same charge state. Protonated BQ is an effective reagent for manipulating charge states of oligonucleotide anions because it induces little or no fragmentation and leads to minimal, if any, adduct ion formation. Furthermore, this ion is high enough in mass-to-charge ratio that trapping by proxy is not necessary for manipulating high-mass oligonucleotides. Therefore, it enables the use of the ion parking technique for oligonucleotide anions, as demonstrated in this work. Protonated peptides tend to show extensive adduction to oligonucleotide anions. The many polar moieties along the peptide background apparently make possible multiple non-covalent interactions with oligonucleotides thereby leading to relatively stable adduct ions. Neither the N-terminal amine nor the C-terminal carboxy group appears to be necessary for peptide ion attachment to oligonucleotide anions.

Acknowledgements

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