Charge Effects for Differentiation of Oligodeoxynucleotide Isomers Containing 8-oxo-dG Residues

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Dissociation reactions of a series of multiply charged oligodeoxynucleotide (ODN) 12-mer anions were studied using an ion trap mass spectrometer. These mixed nucleobase 12-mers fragment first by loss of a neutral nucleobase (A, G, C, and/or 5-methyl-cytosine) followed by cleavage at 3' C–O bond of the sugar from which the base is lost to produce the complementary sequence ions, i.e., [a – B] and w type of ions. No detectable loss of 8-oxo-guanine and/or thymine from these 12-mers is observed under gentle collision conditions in the ion trap. The primary loss of a nucleobase and the subsequent backbone cleavage to generate sequence ions strongly depend on the charge state of the parent molecular ion. For low charge states (2- and 3-), product ions due to the loss of a neutral guanine base and related sequence ions are dominant in the tandem mass spectra. However, preferential loss of a neutral adenine becomes the primary reaction channel from the 5- charge state of the molecular ion. Such charge state dependent fragmentation behavior was utilized to determine the site of 8-oxo-dG residue in a series of structural isomers. The position of 8-oxo-dG residue can be simply determined from the fragmentation pattern of 3- charge state, but not of 5- charge state. It is suggested that in addition to specific modification that affects the N-glycosidic bond strength, total charge content of an ODN is an important factor for determining the differential fragmentation behavior. (J Am Soc Mass Spectrom 2002, 13, 195–199) © 2002 American Society for Mass Spectrometry

There has been growing effort to study modified ODNs using mass spectrometry [1–6]. These studies rely completely on accurate mass measurement and the fragmentation behavior of gas phase ODN ions that can be efficiently produced by either electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI). In the case of ESI, the parent ions are often multiply charged. McLuckey and coworkers reported that parent ion charge state plays a major role in determining the primary fragmentation channels of ODN polyanions [7, 8]. For example, loss of a charged nucleobase other than a neutral is observed exclusively from highly charged parent ions as the initial step in the dissociation of ODNs [7]. However, much debate still exits for the initial neutral base loss from low charge state ions [6, 9–12]. For the MS/MS studies of modified ODNs, Vouros et al. reported that there is a strong tendency for the loss of a nucleobase that is modified with an electron withdrawing group [1]. More recently, Reilly and coworkers showed that modification on the sugar moiety could also drastically change the fragmentation behavior of oligonucleotides [6]. To our knowledge, there is no previous report on the fragmentation behavior of ODNs containing an 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG) residue that is one of the dominant forms of DNA damage generated from oxidative processes as well as ionizing radiation [13].

We report here that the primary dissociation reactions of mixed nucleobase 12-mers strongly depend on the identity of the base and the charge state of the precursor ion. Loss of a neutral guanine base and subsequent cleavage of the 3' C–O bond dominates from precursor ions of low charge states (e.g., 2- and 3-), while preferential loss of a neutral adenine will occur from 5- charge state. No loss of 8-oxo-guanine is observed even during multistage tandem mass spectrometric experiments. We demonstrate these findings by determining the sites of 8-oxo-dG residues in a series of structural isomers.

Experimental

The ODNs used in this study are mixed nucleobase 12-mers, each of which consists of three sets of the four

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nucleobases (or their modified forms) present in DNA, i.e., adenine (A), guanine (G) [or 8-oxo-guanine (G*)], thymine (T), and cytosine (C) [or 5-methyl-cytosine (C*)]. The ODN 5'-CAGTTCGAACTG-3' (**1**) was obtained from Integrated DNA Technologies, Inc. (Coralville, IA), while the ODNs 5'-C*AGTTCGAACTG-3' (**2**) and 5'-CAGTTC*GAACTG-3' (**3**) were purchased from Department of Biological Sciences at University of South Carolina. The ODNs 5'-GTCAAGCTTGAC-3' (**4**), 5'-G*TCAAGCTTGAC-3' (**5**), 5'-GTCAAG*CTTGAC-3' (**6**), 5'-GTCAAGCTTG*AC-3' (**7**), and 5'-GTCAAGC* TTGAC-3' (**8**) were procured from Midland Certified Reagent Company (Midland, TX). These ODNs were purified using either polyacrylamide gel electrophoresis or ion exchange high performance liquid chromatography and were further desalted by passing through a size exclusion desalting column. After purification and desalting, the oligomers were lyophilized and reconstituted in nanopure water and acetonitril (50:50) at a final concentration of 10–20 pmol/μL prior to mass spectrometric analysis.

All experiments were carried out using a Finnigan (San Jose, CA) LCQ ion trap mass spectrometer with a home-made ESI source operated in the negative ion mode. The sample solutions were introduced via direct infusion at 0.5–1.0 μL/min. through a fused-silica capillary tubing with a hand pulled tapered tip at the exit. The electrospray high voltage was 2.0–2.2 kV. The temperature of the heated capillary was at 200 °C. Typically 20–50 scans were averaged for each spectrum shown in this paper. Normalized collision energies for the CID experiments are listed in the corresponding figure legends.

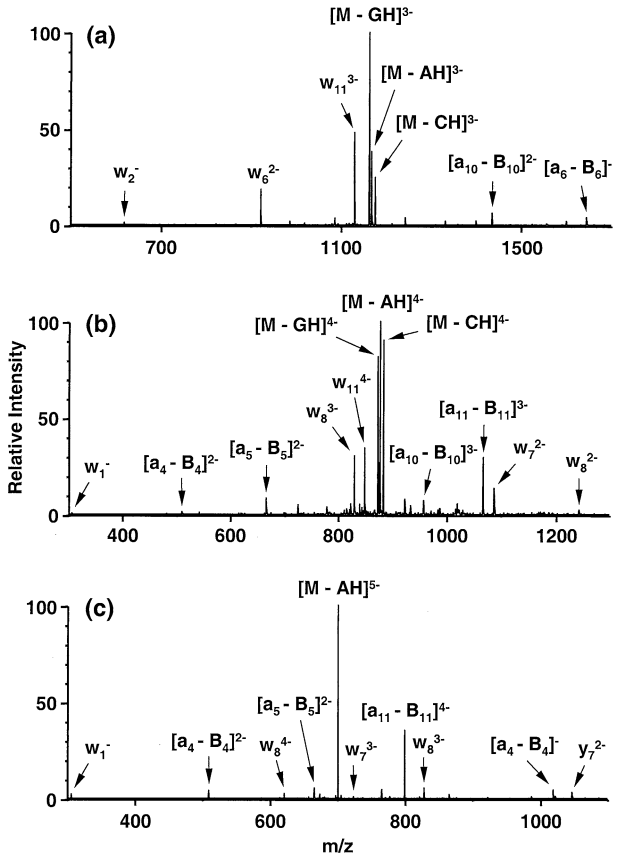

Figure 1. CID mass spectra of the ODN (**4**) from its 3- (**a**), 4- (**b**), and 5- (**c**) charge states at 16%, 18%, and 25% normalized collision energies, respectively.

Results and Discussion

Charge State Effects on Fragmentation Behavior of ODNs

The negative ESI mass spectra of these mixed nucleobase 12-mers exhibit a distribution of multiply charged molecular ions with few adduct ions present. The charge state distribution typically covers a range from 2- to 6- with a maximum at 4- charge state, although the exact number of charge states and the location of the maximum can vary depending on the pH [14] and the concentration of counter ions in the solution [15].

Figure 1 shows the CID spectra of the ODN (**4**) from its 3-, 4-, and 5- charge states. Product ions are assigned according to the nomenclature originally proposed by McLuckey et al. [16]. Loss of a neutral guanine base is the most facile fragmentation channel from the 3- precursor ion (Figure 1a). The two pairs of complementary ions $w_2^-/[a_{10} - B_{10}]^{2-}$ and $w_6^{2-}/[a_6 - B_6]^-$ are indicative of the cleavage of guanine at position 10 and 6, while the ion w_{11}^{3-} suggests the cleavage of the guanine at position 1. Losses of adenine and cytosine are also observed. These cleavages occur at all the adenine sites and at C3 and C7 sites as indicated by the complementary pairs of w and [a – B] type of ions (although their intensities are very low in the mass spectrum). Figure 1b shows the CID spectrum of 4- charge state of (**4**). Essentially all the fragmentation channels revealed from the 3- charge state are also observed from the 4- charge state. However, the relative abundance of the product ions has changed dramatically. In particular, the product ion from the loss of an adenine and sequence ions derived from such a loss become the dominant reaction channels. The major ions shown in Figure 1c from the 5- charge state are dominated by $[M - AH]^{5-}$ and the corresponding sequence ions resulting from loss of a neutral adenine. Note that within the relative errors of the measurements (see below), changing the normalized collision energies (15–25%) did not alter the relative abundances among the different reaction channels.

The charge state dependent fragmentation behaviors are clearly illustrated in Table 1. In Table 1, the product ion abundances are normalized over all sequence ions generated from the loss of G, A, and C at each charge state. These data are average results of all eight 12-mers studied. It is not surprising that the relative errors of the measurement for the low abundant reaction channels are significantly large (50–70%), but the relative errors for the most abundant reaction channels are around

Table 1. Normalized ion abundance of all sequence ions generated from the loss of G, A, and C reaction channels at different charge states

	Reaction channel		
Charge state	G	A	C
3-	68 (64)*	19 (21)	12 (15)
4-	17 (23)	65 (54)	18 (23)
5-	1 (1)	88 (91)	11 (8)

*Values including non-sequence ions are listed in the parenthesis.

10%. Similar results are obtained when nonsequence ions are included in this tabulation. The sequence information contained in the product ion spectrum of each charge state is clearly revealed: at the 3- charge state, the most abundant ions are due to the loss of a neutral guanine, however, little sequence information can be obtained from loss of a guanine at 5- charge state. In contrast, the propensity of losing an adenine from these 12-mers increases for higher charge state and becomes the predominant reaction channel for the 5- charge state. Note the simplicity of the mass spectra of Figure 1a and c compared with Figure 1b. Figure 1b shows more sequence ions at low intensities, whereas Figure 1a and c provide clear information for specific reaction channels: Reaction channels due to loss of a guanine is evident from the CID spectrum of 3- charge state, while reaction channels due to loss of an adenine is readily revealed from the CID spectrum of 5- charge state. Such charge state dependent fragmentation behaviors are very useful characteristics for mapping out damaged residues in ODNs as demonstrated in the following section.

Positional Mapping of 8-oxo-dG Residue in ODNs

The CID spectra of the ODNs containing an 8-oxo-dG residue at positions 1, 6, and 10, i.e., 12-mers (**5**), (**6**), and (**7**), from 3- charge state are shown in Figure 2.Again, the major reaction channels at 3- charge state are due to loss of a guanine base. Interestingly, no loss of 8-oxo-guanine is observed, and so are the related sequence ions. Additionally, no losses of 8-oxo-guanine and thymine are observed even in a multistage tandem mass spectrometric experiment when product ions, for example $[a_{10}–B_{10}]^{2-}$, are selected as the precursor ions for the next stage CID experiment. These are very useful characteristics for determining 8-oxo-dG residues in ODNs since 8-oxo-guanine bases are retained on the product ions, in other words, the damage information is preserved for multiple stage mass spectrometric analysis. In addition, the location of 8-oxo-dG residue can be easily determined from the CID spectra. For example, the CID spectrum of ODN (**5**) which was incorporated an 8-oxo-dG residue at the 5' end (i.e., position 1) showed the absence of the sequence ion of w_{11}^{3-}, while the sequence ion pairs, $w_2^-/[a_{10}–B_{10}]^{2-}$ and $w_6^{2-}/[a_6–B_6]^-$ due to loss of an unmodified guanine base at

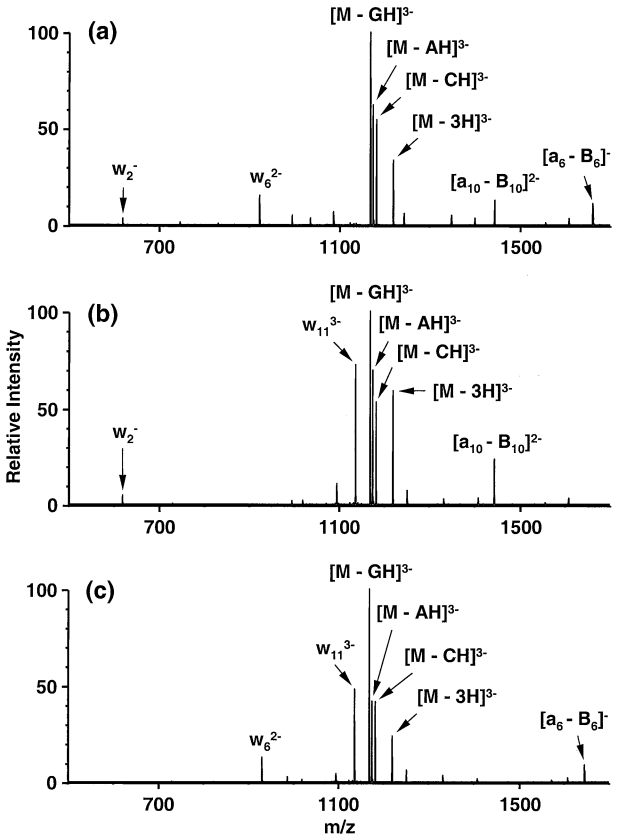

Figure 2. CID mass spectra of the ODNs containing 8-oxo-dG from 3- charge state at 15% normalized collision energy. Spectra of (**a**), (**b**), and (**c**) are for ODNs (**5**), (**6**), and (**7**), respectively.

position 10 and position 6 respectively, are present. Similarly, CID spectra of 12-mers (**6**) and (**7**) showed absence of the sequence ions derived from loss of an 8-oxo-guanine base at their corresponding sites (see Figure 2b and c). Consequently, the position of 8-oxo-dG residue can be revealed by one or two position specific product ion(s) in the CID spectra without requiring many stages of CID to pinpoint the site of the damaged residue in ODNs. In contrast, the CID spectra from 5- charge state of these 8-oxo-dG containing oligomers showed an almost identical fragmentation pattern (see Figure 3). All the major product ions are derived from loss of an adenine. It is evident that the positions of 8-oxo-dG residues cannot be determined from these major product ions. Furthermore, the mass values of the major product ions in Figure 3b and c are the same, leaving no information for even distinguishing these two structural isomers (**6**) and (**7**).

The lack of fragmentation at 8-oxo-dG residue may be due to the inductive effect of the 8-hydroxyl group on stabilizing the N-glycosidic bond. Particularly, the ortho position of the hydroxyl group to the N-glycosidic bond on an aromatic system makes the inductive effect more pronounced. Much higher stability of 8-oxo-dG compared with 2'-deoxygunosine (dG) is also observed in the aqueous solutions [17]. Similar structure feature (considering the tautomeric form) is present in

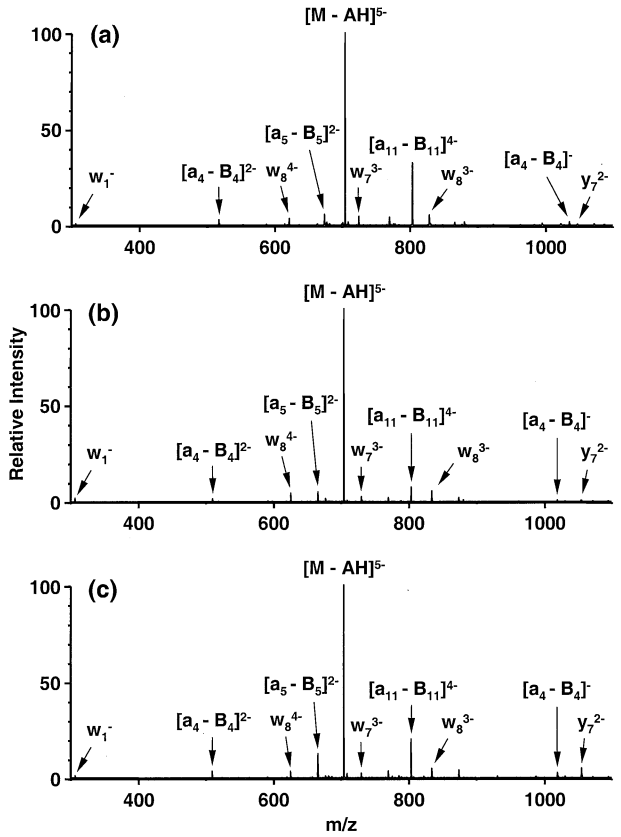

Figure 3. CID mass spectra of the ODNs containing 8-oxo-dG from 5- charge state at 20% normalized collision energy. Spectra of (**a**), (**b**), and (**c**) are for ODNs (**5**), (**6**), and (**7**), respectively.

2'-deoxythymidine (dT), in agreement with the fact that loss of T is rarely observed in mixed nucleobase ODNs [10–12, 18, 19] under gentle collision conditions.

Several mechanisms have been previously proposed for the fragmentation of ODNs [18, 19]. It is widely recognized that there is a strong correlation between the initial loss of a neutral nucleobase and the gas-phase proton affinities of the nucleobases in mononucleotides. As a matter of fact, the current study shows that the propensity for loss of a neutral nucleobase at a low charge state (e.g., 3- charge state) falls in the order of their proton affinities, i.e., G > C ≈ A ≫ T [20]. However, such a correlation fails to account for the dramatic change in the initial loss of a neutral nucleobase at different charge states. Nor does any one of these previously proposed mechanisms [18, 19] fully explain these observations.

We note that the total charge content is an important factor in determining the initial loss of nucleobases from mixed nucleobase ODNs. If one takes an ODN with all the protons detached from the phosphate moieties as being 100% charged, then those ODNs in our experiments at 3-, 4-, and 5- charge states correspond to being 27%, 36%, and 45% charged, respectively. With this consideration in mind, our results are consistent with those obtained from other laboratories using mixed nucleobase ODNs of different lengths and different sequences [18, 19]. For example, Wan and Gross reported CID results from doubly charged ODNs with sequences of 5'-TTAGTT-3' and 5'-TTGATT-3' [18]. Both of these precursor ions are 40% charged and both yield dominant loss of a neutral adenine base and the related sequence ions. Furthermore, they showed that the most abundant sequence ions generated from doubly charged 5'-TTTAGTTT-3' and 5'-TTTGATTT-3' [18], which are 29% charged, are from the loss of a neutral guanine base similar to our results obtained from 3- charge state of the 12-mers.

It is not yet fully understood why loss of a nucleobase is favored over another during the fragmentation of ODNs, and in particular, how the total charge content of ODNs differentially affects their fragmentation. It is possible that several mechanisms [18, 19] may operate depending on the identity of the base, the type of modification, and the charge content. The sequence of an ODN seems to play less of a role in affecting the most dominant fragmentation channels. Nevertheless, the charge state dependent fragmentation behavior is very useful for determining the 8-oxo-dG residue in ODNs using tandem mass spectrometry because the dynamic range of the product ion spectrum can be effectively enhanced by accessing, very conveniently, the sequence informative reaction channels.

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