Fragmentation of Photomodified Oligodeoxynucleotides Adducted with Metal Ions in an Electrospray-Ionization Ion-Trap Mass Spectrometer

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We report the effect of metal–ion adduction on the fragmentation of oligodeoxynucleotides (ODNs) bearing DNA photoproducts. When protons on backbone phosphates of ODNs are completely replaced with metal ions, cleavages occur readily within the photoproduct moiety, whereas those cleavages do not occur in photomodified ODNs in which the phosphates are associated with protons. For example, thymine/adenine (TA*) photoproducts revert to their undamaged precursors upon collisional activation, the pyrimidine(6-4)pyrimidone product and its Dewar valence isomer show a characteristic neutral loss of $C_4H_3NO_3$, and dimeric adenine photoproducts show a distinctive loss of NH_2CN from the adenine six-membered ring. The product–ion mass spectra of photodamaged ODNs that are adducted to metal ions are complementary in terms of structure information to those spectra of ODNs in which the phosphates are associated with protons. The results also demonstrate that the energy required for strand cleavages is higher for ODNs adducted with metal ions than that for ODNs bound with protons. Furthermore, the loss of a pyrimidine is more favorable than the loss of a purine in the fragmentation of ODNs associated with metal ions. (J Am Soc Mass Spectrom 2001, 12, 1174–1179) © 2001 American Society for Mass Spectrometry

Most applications of tandem mass spectrometry (MS/MS) involve using proton-associated ions as precursors. New opportunities arise, however, by using ions that are adducted with metal ions, as was reviewed earlier [1]. Recently, metal ion-associated precursors have been proposed as sources of structural information of oligosaccharides [2–4] and peptides [5–8]. Furthermore, those precursors have been used as a basis for the quantitative measurement of isomeric mixtures by MS/MS [6, 8, 9].

We recently described the unique fragmentations of oligodeoxynucleotides (ODNs) when their backbone phosphates are adducted with metal ions and completely depleted of protons [10]. Those metal-adducted ODNs decompose to give a nearly complete series of sequence ions unlike the corresponding proton-associated ODNs, which do not cleave at T sites. In the fragmentation, the DNA base is lost after a proton is transferred from the C2' of sugar rather than from a backbone phosphate. As a consequence of the mechanism change, the collision energy required for the fragmentation of an ODN increases when the phosphates in an ODN are adducted with metal ions rather than with protons [10].

DNA photoproducts, the cis-syn cyclobutane pyrimidine dimer ([c,s]), the pyrimidine(6-4)pyrimidone product ([6-4]) and its Dewar valence isomer [11, 12], the thymine-adenine photoproduct (TA*) [13, 14], and the dimeric adenine photoproducts (i. e., A=A and (AA)*) [15, 16] are produced upon ultraviolet (UV)-light irradiation (structures of those photoproducts are shown in Scheme **1**). Most of them have been implicated in cell lethality, mutagenesis, and carcinogenesis [12, 17–19]. Some of these materials are isomers, others are only subtly different from their undamaged precursors. Hence, structure determination of these photoproducts is a particular challenge for mass spectrometry. We wish to develop methods that meet the challenge because mass spectrometry offers high sensitivity in the investigation of these materials, and MS/MS provides structural information.

In a recent article [10], we showed that the product–ion spectra of $[M + 4Na - 6H]^{2-}$ of d(GTATTAT) and d(GTAT[c,s]TAT) are distinguishable from each other, whereas the product–ion spectra of $[M - 2H]^{2-}$ of the two isomers are not [20]. Here we report an extension of that study whereby we investigate the effect of metal–ion adduction on the fragmentation of a set of photomodified ODNs that we prepared [20, 21]. We wish to continue our investigation of the strengths and weak-

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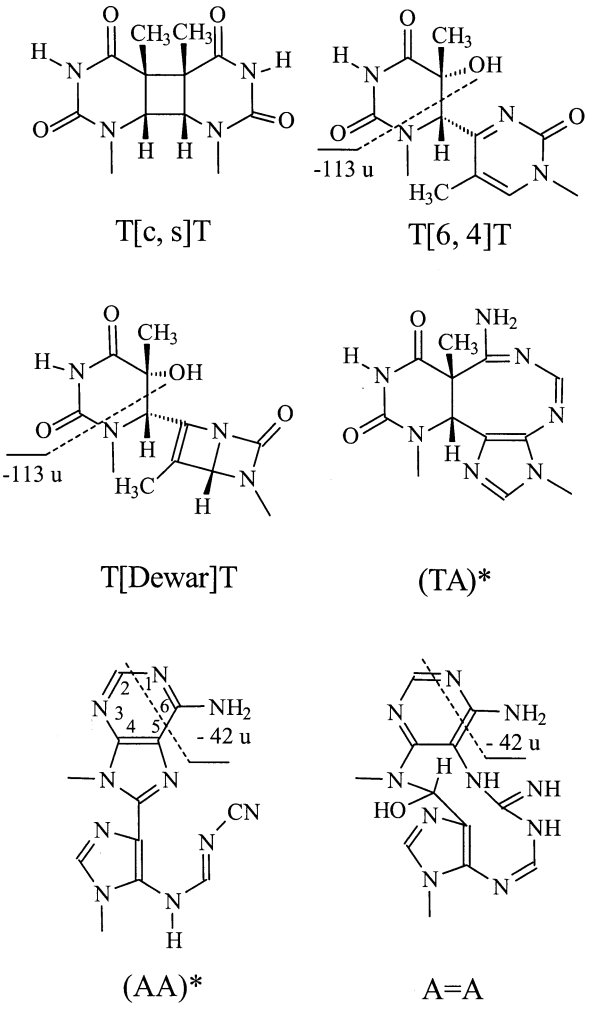


Scheme 1

nesses of the utility of metal ions in the structure elucidation of various ODNs.

Experimental

Oligodeoxynucleotides

ODNs in this study were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and used without further purification.

All photomodified ODNs except d(GTAT[Dewar]TAT) were obtained by 254-nm irradiation of ODNs followed by high-performance liquid chromatography (HPLC) separation as described in previous studies [20, 21]. ODNs were dissolved in doubly distilled water and degassed with N_2 for 5 min. The solutions were dispersed in petri dishes with an internal diameter (i.d.) of 10.2 cm; the dishes were then transferred into a N_2-filled, zip-lock bag, the solution was irradiated on ice for 2 h, and the mixture dried with a Savant Speed-Vac (Savant Instruments Inc., Holbrook, New York). To prepare d(GTAT[Dewar]TAT), 50 nmol of d(GTAT[6-4]TAT) was dissolved in 500 μL water, transferred into a NMR tube, and irradiated with medium-pressure mercury arc light for 30 min as described previously [22, 23]. Under this irradiation condition, the [6-4] product isomerized quantitatively to its Dewar valence isomer [22, 23]. All solutions were dried under vacuum in a Speed-Vac, and the resulting products were used without further purification.

The HPLC separations were done by using a Dynamax 4.6* 250-mm reverse-phase C18 column with a particle size of 5 μm and a pore size of 300 Å (Varian, Walnut Creek, CA). Two solutions were used for separation; solution A was an aqueous solution of 50 mM triethylammonium acetate (pH 7.0), and solution B was 30% acetonitrile in A. A 40-min linear gradient of 20–40% buffer B was employed, and the flow rate was 1.0 mL/min. The HPLC elution times and the structure assignments of those photoproducts were reported previously [20, 21].

Electrospray Mass Spectrometry on an Ion-Trap Instrument

Mass spectrometry (MS) experiments were carried out on a Classic LCQ ion-trap mass spectrometer (Finnigan, San Jose, CA) with an electrospray-ionization source. A solution of 50/50 (vol/vol) of acetonitrile/H_2O was used as the electrospray solvent. ODNs were dissolved in doubly-distilled water for spraying when product-ion spectra of $[M - 2H]^{2-}$ ions were to be obtained, and in 2 mM NaCl or LiI aqueous solution when those of the sodium and lithium ion-adducted species were to be obtained. Under those conditions, the fully metal–ion associated species is the major component, as determined by the full-scan electrospray ionization (ESI) mass spectra, but partially metal–ion associated species were also present. The total ion current decreased by 2- to 3-fold compared to that produced when an ODN solution without metal–salt addition was sprayed. A 5 μL aliquot of a 10–20 μM sample solution was injected for each run. The spray voltage was 4.6 kV, and the capillary temperature was 200 °C.

Tandem mass spectra were obtained by selecting the deprotonated, $[M - H]^-$, $[M - 2H]^{2-}$, or the appropriate metal-adducted ions for collisional activation; the mass width for precursor selection was 3 *m/z* units, and the collision gas was helium. The resonance excitation voltage for MS/MS experiments was 42% of the 5 V peak-to-peak maximum voltage available on the instrument; the *q* value for activation was 0.25, the activation time was 30 ms, and product ions were cooled for at least 2 ms before detection. Each spectrum was an average of approximately 30 scans, and the data acquisition time for each scan was 1.0 s.

Nuclease P1 Digestion

Nuclease P1 was procured from Boehringer-Mannheim (Mannheim, Germany) and used without further puri-

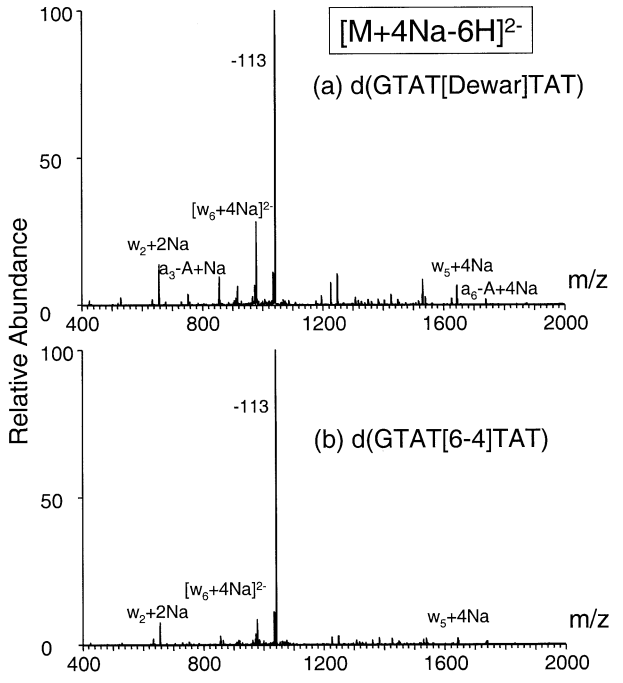


Figure 1. Product–ion spectra of ESI-produced $[M + 4Na - 6H]^{2-}$ ions of (**a**) d(GTAT[Dewar]TAT), and (**b**) d(GTAT[6-4]TAT).

fication. For all the digestion reactions, no additional buffer was used except that present in the commercial preparation of the enzyme. In a typical digestion reaction, 0.5 μL of 1 unit/μL nuclease P1 solution (units of enzyme as defined by the vendor) was added to 25 μL of 10 μM photomodified ODN, and the solution was incubated at 37 °C for 5 min. The resulting digestion sample was injected directly for mass spectrometric analysis.

Results and Discussion

ODNs Bearing Pyrimidine(6-4)Pyrimidone Product and its Dewar Valence Isomer

We showed previously [10] that an ODN, whose backbone phosphates are completely depleted of protons, fragments to give a product–ion spectrum containing an almost complete series of sequence ions no matter if the ODN is T-rich or of mixed sequence. From those sequence ions, we were able to distinguish d(GTAT[c,s]TAT) from the undamaged d(GTATTAT) [10]. In an effort to show whether other photodamaged ODNs that are associated with metal ions give distinctive fragmentation, we investigated the product–ion spectra of the $[M + 4Na - 6H]^{2-}$ ions of d(GTAT[6-4]TAT) and d(GTAT[Dewar]TAT) (Figure 1). These isomers can be distinguished from those of d(GTATTAT) and d(GTAT[c, s]TAT). The most predominant feature of the two product–ion spectra is the production of an abundant fragment ion of *m/z* 1041 (Figure 1), which arises by a neutral loss of 113 u [presumably $C_4H_3NO_3$ from the 5'-modified base in the (6-4) product]. This neutral loss also occurred upon collisional activation of $[M - H]^-$ of d(pT[6-4]TA) and d(pT[Dewar]TA) [24]. Interestingly, the proportion of this fragment ion with respect to the total ion flux from d(GTAT[Dewar]TAT) is not as high as that from d(GTAT[6-4]TAT), which is consistent with the observation that the loss of 113 u does not occur as readily for $[M - H]^-$ of d(pT[Dewar]TA) as it does for that of d(pT[6-4]TA) [24].

The process giving an ion by loss of 113 u ion is only facile for the $[M + 4Na - 6H]^{2-}$, and is not detectable in the fragmentation of $[M - 2H]^{2-}$ of the two isomers [20]. Presumably, the energy required for the loss of 113 u is higher than that for the strand cleavage of $[M - 2H]^{2-}$ (i.e., for the process leading to the formation of w_n and $[a_n - Base]$), but lower than that for the strand cleavage of $[M + 4Na - 6H]^{2-}$. This result is in accord with the conclusion that the energy required for the fragmentation of an ODN is higher when it is adducted with metal ions than with protons [10].

In a previous study [24], we showed that nuclease P1 can degrade a photomodified ODN to smaller fragments bearing the photoproduct (i.e., a trinucleotide provided the photomodification is not at either end of the ODN). The collisionally activated dissociations (CAD) of most of those fragments are distinctive. The product–ion spectra of $[M - H]^-$, $[M + Na - 2H]^-$, and $[M + 2Na - 3H]^-$ of d(pT[6-4]TA), which was produced from the nuclease P1 digestion of d(GTAT[6-4]TAT) [24], shows that the loss of 113 u becomes more predominant with the adduction of more metal ions, whereas the ion of *m/z* 803 [M − A], (A is adenine) becomes less abundant (Figures 2a, b, and c). The ions of *m/z* 803 and *m/z* 825 (produced by loss of 113 u) are comparably abundant in the product–ion spectrum of $[M - H]^-$. The process to lose 113 u, however, is much more facile than that to lose adenine from $[M + Na - 2H]^-$. Furthermore, this loss gives the dominant fragment ion, as seen in the product–ion spectra of $[M + 2Na - 3H]^-$. The product–ion spectra of d(pT-[Dewar]TA) show that this species has similar reactivity as d(pT[6-4]TA) (data not shown).

*ODNs Bearing Thymine Adenine Photoproduct (TA)**

We previously showed that $[M - 2H]^{2-}$ of d(GTAT(TA)*T) fragments to a_6 instead of $[a_6 - A]$, which is produced from d(GTATTAT). In the photomodified ODN, the adenine in the sixth position is linked to thymine at the fifth position, and this prevents the loss of the base [20]. The product–ion spectrum of $[M + 4Na - 6H]^{2-}$ of d(GTAT(TA)*T), however, is very similar to that of the undamaged d(GTATTAT). We observed that $[M + 4Na - 6H]^{2-}$ of both d(GTAT(TA)*T) and d(GTATTAT) [Figure 3a shows the product–ion spectrum of d(GTAT(TA)*T)] gives

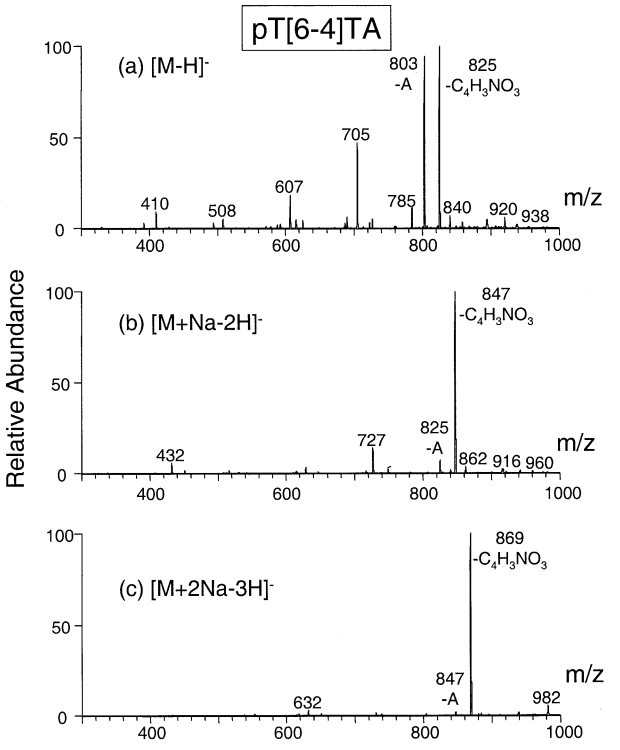


Figure 2. Product–ion spectra of ESI-produced (**a**) $[M - H]^-$ (**b**) $[M + Na - 2H]^-$, and (**c**) $[M + 2Na - 3H]^-$ ions of d(pT[6-4]TA) resulted from nuclease P1 digestion of d(GTAT[6-4]TAT).

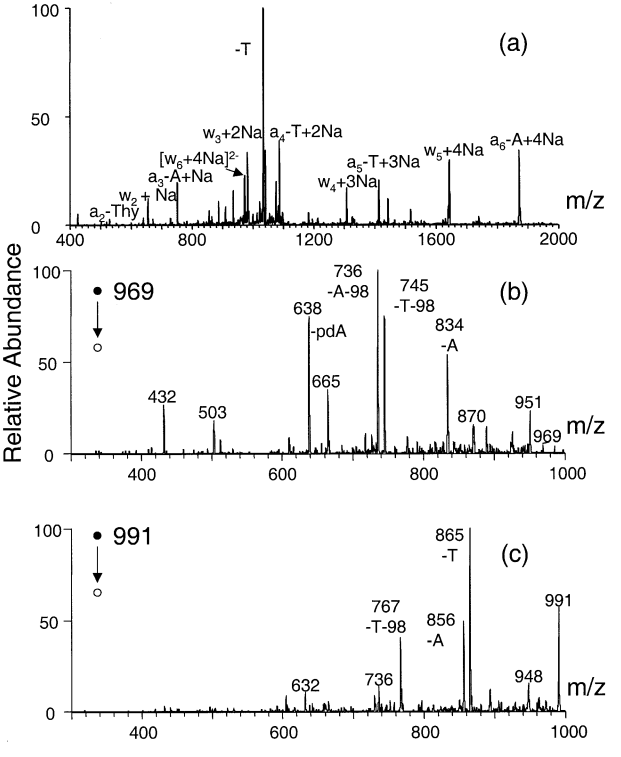
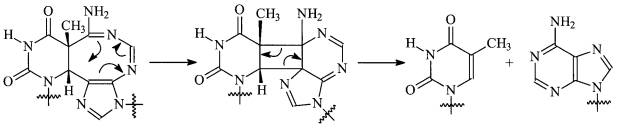


Figure 3. Product–ion spectra of ESI-produced (**a**) $[M + 4Na - 6H]^{2-}$ of d(GTAT(TA)*T), (**b**) $[M + Na - 2H]^-$ ion of d(p(TA)*A), and (**c**) $[M + 2Na - 3H]^-$ ion of d(p(TA)*A). Trinucleotide d(p(TA)*A) was obtained from nuclease P1 digestion of d(GGCTA(TA)*A).

$[a_5 - T + 3Na]$ (T is thymine), $[a_6 - A + 4Na]$ and $[w_2 + Na]$ in their respective product–ion spectra. These three ions must arise by cleavages between the bases that had been joined through the photochemistry, reverting the ODN to an undamaged state. We tentatively propose an intramolecular rearrangement mechanism for the reversion of the photoproduct to give back an ODN with normal bases thymine and adenine. The reversion may also take place via the formation and subsequent cleavage of a cyclobutane ring (Scheme **2**). The product–ion spectrum of $[M + 4Na - 6H]^{2-}$ of d(G(TA)*TTAT) and those of $[M + 5Na - 7H]^{2-}$ of d(GGC(TA)*TAA) and d(GGCTA-(TA)*A) also showed that the (TA)* reverted to an ODN in which thymine and adenine are not connected (data not shown). The product–ion spectra of the photoproducts and the unmodified ODN are nearly identical.



Scheme 2

We also observed the intra-photoproduct cleavage and the reversion of the photoproduct to the undamaged states in the product–ion spectra of $[M + Na - 2H]^-$ and $[M + 2Na - 3H]^-$ of d(p(TA)*T) and d(p(TA)*A), which were obtained from nuclease P1 digestion of d(GTAT(TA)*T) and d(GGCTA(TA)*A), respectively. For example, the loss of thymine (−T and/or −T − 98) occurs from both $[M + Na - 2H]^-$ and $[M + 2Na - 3H]^-$ of d(p(TA)*A) (Figures 3b and c), and the loss of adenine occurs from both $[M + Na - 2H]^-$ and $[M + 2Na - 3H]^-$ of d(p(TA)*T) (spectra not shown).

Unfortunately, the intra-photoproduct reversion reaction makes ineffective, for structure determination, the use of MS/MS of metal-cationized ODNs bearing TA* photoproducts. On the other hand, $[a_5 - T + 3Na]$, $[a_6 - A + 4Na]$ and $[w_2 + Na]$, which are all formed from intra-photoproduct cleavages, are as abundant as other $[a_n - Base]$ and w_n ions. This indicates that the energy for intra-photoproduct cleavage of (TA)* is comparable to that for strand cleavages of the ODN when there is no proton on its phosphates. The observation that the intra-photoproduct reversions do not occur in the $[M - 2H]^{2-}$ ion demonstrates that the energy required for strand cleavage in proton-adducted ODNs is lower than that in metal ion-adducted ODNs.

ODNs Bearing Dimeric Adenine Photoproducts

The product–ion spectra of the $[M + 5Li - 7H]^{2-}$ of d(CGT(AA)*TGA) and d(CGTA=ATGA) show that abundant ions are formed by losses of NH_2CN to give

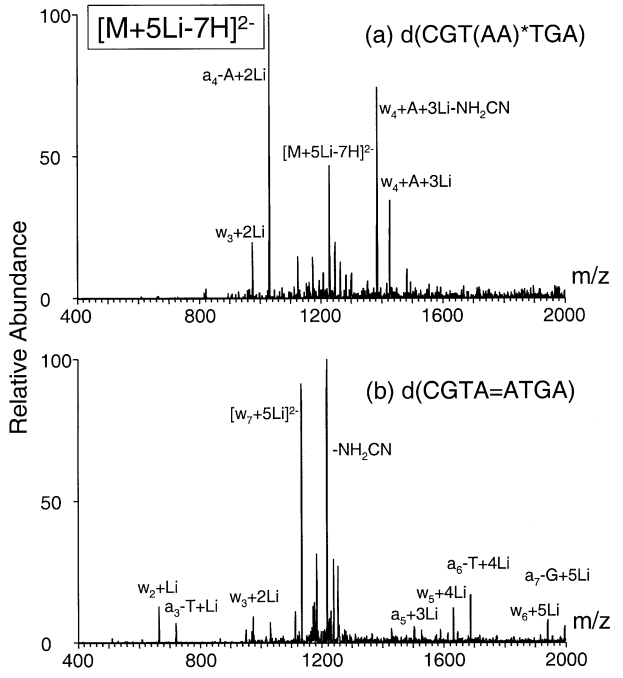


Figure 4. Product–ion spectra of ESI-produced [M + 5Li − 7H]$^{2-}$ of (**a**) d(CGTA=ATGA) and (**b**) d(CGT(AA)*TGA).

[w_4 + A + 3Li − NH_2CN] in the former and [M − NH_2CN] in the latter (Figures 4a and b). The loss of NH_2CN likely originates from the six-membered ring of adenine N1-C6-N^6 (Scheme **1**). Such cleavages do not occur to any extent at an adenine site in an unmodified ODN nor from the [M − 2H]$^{2-}$ of ODNs containing dimeric adenine photoproducts [21].

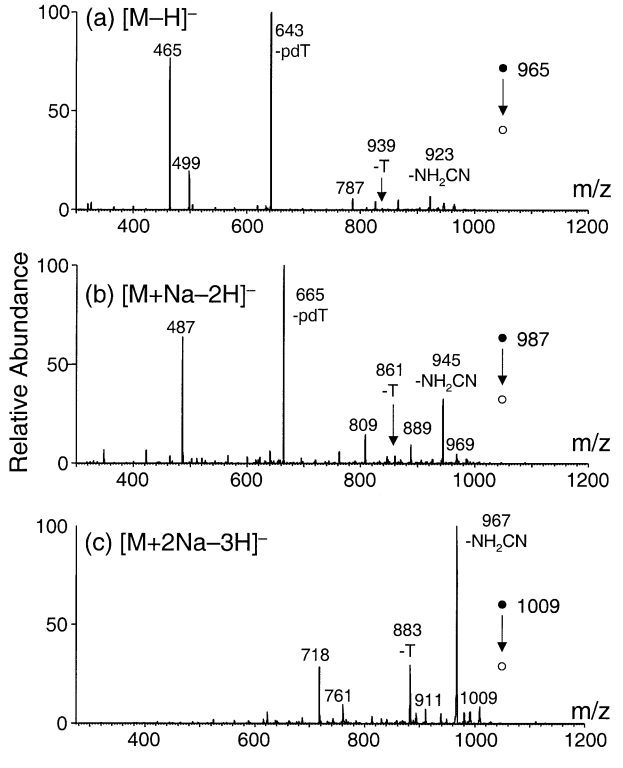


Figure 5. Product–ion spectra of ESI-produced (**a**) [M − H]$^-$, (**b**) [M + Na − 2H]$^-$,and (**c**) [M + 2Na − 3H]$^-$ ions of d(pA=AT), which is extricated from d(CGTA=ATGA) by nuclease P1 digestion.

The product–ion spectra of [M − H]$^-$, [M + Na − 2H]$^-$ and [M + 2Na − 3H]$^-$ of d(pA = AT), which was extricated from d(CGTA=ATGA) by nuclease P1, also show that there is a dramatic difference between the d(pA=AT) adducted with sodium ions compared to those bound with H^+ (Figure 5). In the product–ion spectrum of [M − H]$^-$, we find evidence for the loss of the 3' thymidine (pdT) to give the ion of *m/z* 643 and for further loss of the sugar and phosphate from the 5' modified adenine to give the ion of *m/z* 465 [21]. In the product–ion spectrum of [M + 2Na − 3H]$^-$, the ion of *m/z 967* is seen to be a predominant fragment, the formation of which involves the loss of the familiar NH_2CN group [21]. This ion and the one formed by loss of thymine become more and more abundant as the adduction with sodium ions becomes more complete (Figures 5a, b, and c).

Similarly, the product–ion spectrum of d(p(AA)*T) showed that the loss of the 3' thymidine becomes less facile, whereas that of NH_2CN becomes more facile, as the precursor contains more and more sodium ions (data not shown).

Conclusions

This article is a report of another step in our efforts to explore the effects of metal–ion adduction on ODN fragmentations. Various photomodified ODNs (i.e., ODNs containing T[6-4]T, T[Dewar]T, TA*, (AA)*, and A=A) adducted with metal ions on their backbone phosphates undergo more readily the intra-photoproduct cleavages than when the phosphates are associated with protons. This type of fragmentation is preferred when determining the structure of ODNs with new kinds of photodamage.

The effect of metal–ion adduction is the same for the small fragments from photomodified ODNs digested with nuclease P1; the dispersion of ion signal induced by metal ions in the small fragment is not as severe as that in the intact ODN. Therefore, the combination of nuclease P1 digestion with MS/MS of the products adducted with metal ions should be particularly useful for the structure determination of modified ODNs.

In a previous study [10] we demonstrated from "breakdown" curves that the energy required for the fragmentation of metal–ion adducted ODNs is higher than that of proton-adduced ODNs. More evidence for this phenomenon is that intra-photoproduct cleavages are facile for ODNs adducted to metal ions but not for ODNs associated with protons.

T[c,s]T-containing ODNs give more distinctive fragmentation than their undamaged parents when adducted with metal ions [10]. Distinguishing these materials, however, is not possible by submitting the [M − 2H]$^{2-}$ ions to MS/MS [20]. When ODN phos-

phates are bound with metal ions, the intra-photoproduct reversion reaction makes TA*-containing ODNs indistinguishable from the undamaged molecules. The fragmentations of $[M - 2H]^{2-}$ of the two isomers, however, are distinctive [20]. From the results of our previous studies [10, 20, 21, 24] and those reported here, we conclude that collisional activation of ODNs adducted with metal ions is complementary to that of ODNs associated with protons for the structure determination of photomodified ODNs. We expect that this complementary relationship will extend to modified ODNs in general.

This study also illuminates an interesting trend for base loss in the fragmentation of ODNs. With increasing metal–ion adduction, the loss of thymine from d(pA = AT) occurs more and more easily, whereas the loss of the adenine from d(pT[6-4]TA) becomes less and less facile. This result is in accord with the previous observation that loss of pyrimidines is more facile than that of purines from ODNs adducted with metal ions [10].

Acknowledgments

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