ELSEVIER

International Journal of Mass Spectrometry 190/191 (1999) 303–312

International Journal of Mass Spectrometry

Location of the Na^+ cation in negative ions of DNA evidenced by using MS^2 experiments in ion trap mass spectrometry

Amélie Favre, Florence Gonnet, Jean-Claude Tabet*

Laboratoire de Chimie Structurale Organique et Biologique, CNRS UMR 7613, Université Pierre et Marie Curie, 75252 Paris Cedex 05, France

Received 13 October 1998; accepted 24 February 1999

Abstract

Collision-induced dissociation (CID) spectra of deprotonated molecules prepared from a variety of monocationized homonucleotides and heteronucleotides, using an external nanoelectrospray source with an ion trap mass spectrometer, are described. Under such conditions, the achieved sensitivity in the negative ion mode is sufficient to obtain useful CID mass spectra of deprotonated oligonucleotides from doubly or triply charged negative ions. Results from the analysis of the daughter ions show that the negative charges are located on 5′- or 3′-terminal sites whereas the salt group is at a central position. The cation appears to remain stationary during the CID process. Moreover, evidence exists that the central cation site is neither influenced by the size of the oligonucleotide nor by the nature of the 5′- or 3′-terminus base, which indicates particular stability for the doubly or triply charged quasimolecular anion. (Int J Mass Spectrom 190/191 (1999) 303–312) © 1999 Elsevier Science B.V.

Keywords: Collision-induced dissociation; Na^+ cation; Ion trap mass spectrometry; DNA

1. Introduction

The rapid growth in the use of synthetic oligonucleotides has spurred the development of analytical techniques that can accurately and quickly determine the integrity of the synthetic products. In this way, a number of new ionization/desorption techniques have been developed allowing routine analysis of biomolecules by mass spectrometry, including plasma desorption (PDMS) [1,2], fast atom bombardment (FAB) [3–5], matrix assisted laser desorption ionization (MALDI) [6], and electrospray ionization (ESI) [7,8].

In terms of the fundamental research, understanding the gas phase chemistry of these desorbed ions is important both to obtain information on ion structure and on spacial arrangement, as well as on reactivity toward neutrals. In contrast to the large number of structural data for nucleic acids in solution, there is poor information about conformation of the gas phase ions [9]. Some studies have been reported about proton affinity [10,11] and acidity [12,13] scales of nucleosides and nucleotides, as well as deprotonated DNA oligomer conformations in the gas phase (a 10 thymine nucleotide oligothymidine) [14,15]. D. E. Clemmer and co-workers [15] have measured colli-

* Corresponding author. E-mail: tabet@ccr.jussieu.fr

Dedicated to J.F.J. Todd and R.E. March in recognition of their original contributions to quadrupole ion trap mass spectrometry.

PII S1387-3806(99)00049-4

sion cross sections for different charge states of oligonucleotides, prepared under electrospray ionization mode by using ion mobility mass spectrometry [16]. They showed that the gas phase conformation of these chains strongly depends upon the number of charged sites and their location. Moreover oligonucleotide negative ions containing sodium cation are characterized by higher mobilities than the deprotonated DNA ions of the same charge state. They concluded that binding sodium leads to more compact conformations for lower charge states.

In addition to information regarding molecular weight, tandem mass spectrometry (MS/MS) combined with collision-induced dissociation (CID) may also provide valuable information on the nucleotide sequence. These dissociations can be performed using high and low energy collision regimes in sector and quadrupole instruments [8,17,18], infrared multiphoton dissociation (IRMPD) in FTMS [19,20], or ion trap mass spectrometry. In the latter way, McLuckey and co-workers [21–24] presented CID mass spectra (under resonant excitation conditions) of multiply charged negative oligonucleotides utilizing a coupled ESI external source. Such ion analyzers operate at the low collision energy regime as tandem triple quadrupole mass spectrometers. Both gave rise to formation of similar fragment ions, with an analytical advantage to the former due to sequential MS^n experiments and the accumulation of ions. Furthermore, Hunt and co-workers [25] have shown, by comparison of MS/MS experiments performed on a triple quadrupole and on an ion trap mass spectrometer, that a larger internal energy distribution is observed using ITMS [26].

For DNA analysis, 5 nmol per hour are required for the electrospray technique, which can be considered an analytical limitation. As an alternative, the use of nanoESI [27] coupled with a quadrupole ion trap [28] has been a successful method for performing our experiments with high sensitivity. This allows the analysis of small amounts of samples (25 pmol consumed per hour) and a reasonable achieved sensitivity because of the possibility of long accumulation time in the ion trap cell. The nanospray mode is a powerful method in the negative ion mode, because small voltages were applied (compared to ESI) to reach a stable spray, avoiding the well-known Corona discharges. This technique allows for analysis conditions that are the most representative of the biological environment.

The aim of this work is to locate the position of one sodium cation on a deprotonated oligonucleotide strand. This work was based upon the assumption that a Na^+ cation appears to be less mobile than a proton [29]. This behavior can be rationalized by considering that the sodium cation is strongly bound to alkoxyde (PO^- Na^+) unlike the proton that is covalently linked to the alkoxyde charged site (PO-H). It is important to know whether the Na^+ cation is located or not because this allows study of the origin of single strand conformation. This information is achieved using sequential CID mass spectra of negative precursor ions, as synthetic oligonucleotides having different lengths, sequences (homo or hetero base constitutions), and structures (dephosphorylated or phosphorylated at the 5′ end).

2. Experimental

2.1. Instrumentation

All these experiments were performed on an Esquire ion trap (Bruker-Franzen Analytic GmbH, Bremen, Germany) [30]. The standard scan corresponds to the mass-to-charge ratio analytical scan from 50 Th to 2000 Th, by selective resonant ejection of ions using the nonlinear resonance at $\beta_z = 2/3$ ($q_z = 0.78$). Axial modulation is also applied to enhance ion resolution. The analytical scan rate was approximately 2000 Th/s. No ion current control (ICC) was used, which explains the *m/z* scale decalibration. The ion trap was operated at an estimated buffer He gas bath pressure of 1.8×10^{-5} Torr (2.35×10^{-3} Pa). A differentially pumped Analytica interface (Analytica of Branford, Inc., Branford, CT) transfers the ion jet from the external NanoESI source, through the transfer capillary, to the mass spectrometer via an rf-only hexapole.

In the MS/MS mode, the ion isolation step was

performed by the application of a broadband ejection of ions, with an ion isolation window of 4 or 6 Th width. This was chosen in order to increase the sensibility and to avoid selected ion ejection or premature dissociation. Each ESI mass spectrum was recorded in the negative ion mode by an average of 10 mass spectrum summations, within the 200–2000 Th range, under a skimmer voltage of −15 V, and an accumulation time of 200 ms. For collision-induced dissociation (CID) of the selected ions, a resonant excitation frequency with an amplitude of 0.5–1.7 V was applied during 40 ms on a window of 10 Th width. The used low *m/z* cutoff of fragmentation was approximately 1/3 the *m/z* value of the selected ion.

The electrospray source was replaced by the NanoESI source purchased from European Molecular Biology Laboratory (Heidelberg, Germany) [28]. NanoESI capillaries were purchased precoated from the Protein Analysis Company (Odense, Denmark). The sample solution (1–10 μL) is loaded into the needle with a thin gel-loader tip. The air-tight stainless steel capillary allows application of an arbitrary air pressure below 1 bar to keep the spray constant. The needle is centered 1–2 mm in front of the orifice, coaxially to the mass spectrometer orifice. A voltage of −500 V is sufficient to initiate the spray because of the small distance between needle tip and orifice. A metal cap was supplied for the entrance transfer capillary. This cap slid over the transfer capillary and fit tight with an O-ring seal. Indeed, the orifice size of the transfer capillary was reduced from the original 500 μm to 200 μm without an apparent decrease in ion current. Moreover, no N_2 counterflow was needed in NanoESI, owing to the small flow rate (30 nL/min) [27]. A new precoated glass capillary was used for each experiment to avoid contamination and removal of the coating from the sample capillary.

2.2. Chemicals

All samples, at a concentration of 5 pmol/μL, were dissolved in methanol/H_2O (50/50; v:v) with 10 mM triethylammonium acetate.

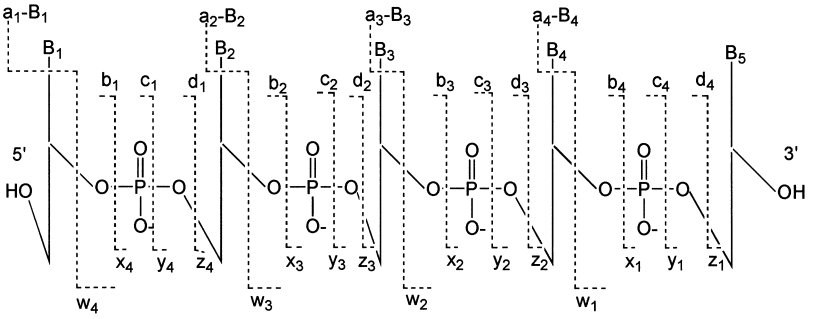
The 5′-phosphorylated oligonucleotides $dp(A)_7$ (M = 2209.4 u) and $dp(T)_7$ (M = 2146.3 u) were purchased from Sigma (St. Louis, MO), and the 5′-dephosphorylated oligonucleotides such as d(ACCCAAACCAA) (M = 3261.6 u), d(TTGGTTTGGGT) (M = 3407.6 u), d(TTGGCCAA) (M = 2408.5 u), and d(TTAGCTAA) (M = 2407.5 u) were synthesized at the 100 μmol range, using phosphoramidate chemistry on a solid support (Milligen 8800) [31]. The purity was monitored by capillary electrophoresis and analytical high-performance liquid chromatography (HPLC) by T. Huynh Dinh (Institut Pasteur, Paris, France). Methanol was purchased from Prolabo (HPLC purity). Desalted water was of Milli-Q grade.

No Na^+ cation was added to perform the following experiments. Sodium cations originated from the solvents and were incorporated into the DNA single strands.

3. Results and discussion

Collision-induced dissociation mass spectra of oligonucleotide negative ions have been shown to be effective in yielding extensive sequence information for single strand DNA oligomers. The nomenclature of oligonucleotide fragment ions is based upon the work of McLuckey [21] (Scheme 1). Furthermore, in order to simplify the writing of the multiply charged $[M\text{-}xH]^{x-}$ ion, the $[M]^{x-}$ notation is used. In the same way, $(a_i\text{-BH-}xH)^{x-}$, and $(a_i\text{-}xH)^{x-}$ ions are noted, respectively, as $(a_i\text{-BH})^{x-}$ and $(a_i)^{x-}$. Moreover, when H^+ is exchanged with Na^+, the writing of $(a_i\text{-BH-}xH + Na)^{(x-1)-}$ is simplified to $*(a_i\text{-BH})^{(x-1)-}$.

To obtain information about the orientation of DNA polyanion fragmentations, several assumptions will be considered as sodium cation confining on DNA single strand oligomers and negative charge location on acidic forms. In this way, different partially deprotonated oligonucleotides exchanging one proton against one Na^+ have been studied under resonant excitation after precursor ion selection. Because the sodium cation does not migrate prior to and/or during fragmentation [29], its location can be reached by direct interpretation of the observed product ions of the selected parent $*[M]^{(x-1)-}$ ions.



Scheme 1.

The first two ESI mass spectra studied were obtained from $dp(A)_7$ and $dp(T)_7$. These oligonucleotides contain a 5′-phosphodiester group. On the ESI mass spectrum of $dp(A)_7$ (data not reported herein), not only is the doubly charged M^{2-} ion at *m/z* 1104 displayed, but also the sodium salt doubly charged *m/z* 1116 ion. After isolation of this triply deprotonated cationized *m/z* 1116 ion, CID experiments are performed [Fig. 1(a)]. The loss of adenine as a neutral constitutes the first cleavage, yielding the $*[M\text{-}AH]^{2-}$ ion that is displayed at *m/z* 1047 in Fig. 1(a). Moreover, the cleavage at the 3′ C–O bond of the deoxyribose from which the nucleobase has been lost, leads to the complementary $*w_i^-$ and $(a_{n-i}\text{-}BH)^-$, and w_{n-i}^- and $*(a_i\text{-}BH)^-$ ion types via proton transfer.

The fragment ions generated from the parent $*M^{2-}$ ions of $dp(A)_7$ and $dp(T)_7$ are summarized in the corresponding CID spectra [Fig. 1(a) and 1(b)].

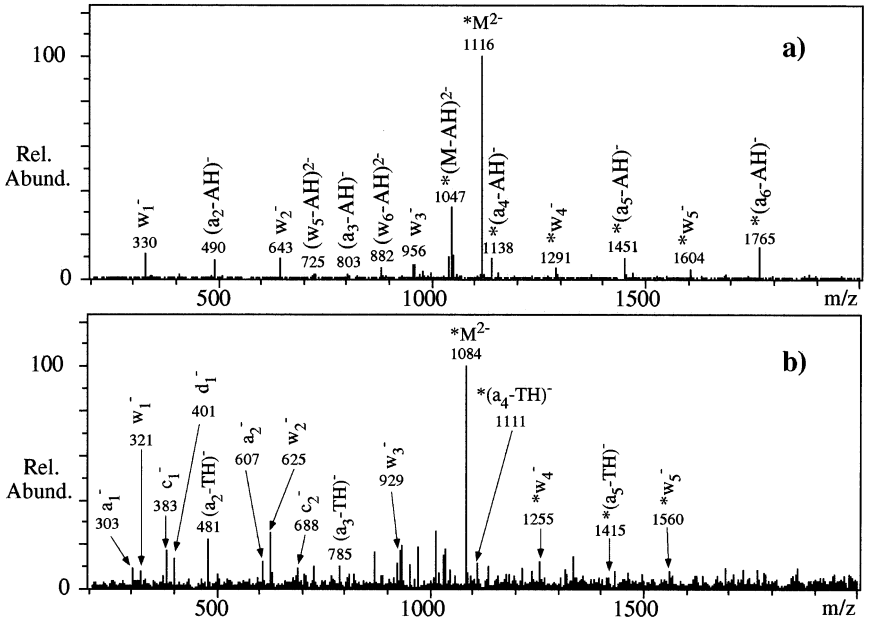
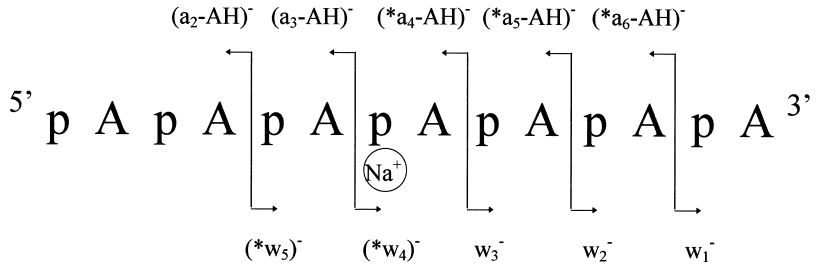


Fig. 1. CID spectra of negative ions prepared under ESI conditions, under a capillary exit voltage of −150 V, and a low *m/z* cutoff value of 200 Th, recorded after the selection of (a) *m/z* 1116 ion ($*[M]^{2-}$) of d(pApApApApApApA), with a *m/z* window of 4 Th and an ion excitation with a resonant frequency amplitude of 1.2 V and a low *m/z* cutoff value of 309 Th; (b) *m/z* 1084 ion ($*[M]^{2-}$) of d(pTpTpTpTpTpTpT), with a *m/z* window of 4 Th and an ion excitation with a resonant frequency amplitude of 1.6 V and a low *m/z* cutoff value of 301 Th, to be to similar q_z.



Scheme 2.

For instance, from $dp(A)_7$, instead of the w_i^- ion type series, the cationized fragment $*w_5^-$ and $*w_4^-$ ions are observed at *m/z* 1604 and *m/z* 1291. This points out an *m/z* shift of 22 Th versus the expected *m/z* values (i.e. *m/z* 1583 and *m/z* 1270) for the w_5^- and w_4^- ions. This corresponds to the exchange of one hydrogen by one sodium cation in singly charged ions [Fig. 1(a)]. Thus, the produced fragment ions preserve the sodium cation whereas the free-of-cation-fragment w_3^-, w_2^-, and w_1^- ions, respectively, appeared at *m/z* 956, *m/z* 643, and *m/z* 330. This suggests that the sodium cation should be located at the fourth phosphodiester linkage, owing to the hypothesis that Na^+ migration cannot take place prior to or/and during fragmentation [29]. Furthermore, if the $(a_i\text{-BH})^-$ ion type series is considered, the daughter $*(a_6\text{-AH})^-$, $*(a_5\text{-AH})^-$, and $*(a_4\text{-AH})^-$ ions are displayed at *m/z* 1765, *m/z* 1451, and *m/z* 1138, respectively. They correspond to Na^+ cationized species, because the free-of-cation ones are theoretically expected at *m/z* 1743, *m/z* 1430, and *m/z* 1117. If these fragment ions carry the sodium cation, it is not the case for the fragment $(a_3\text{-AH})^-$ and $(a_2\text{-AH})^-$ ions observed at *m/z* 803 and *m/z* 490. This gives direct evidence for the confinement of the sodium on the fourth phosphodiester linkage. If w_i^- ion type series and $*(a_{n-i}\text{-BH})^-$ ion type series (or/and $*w_{n-i}^-$ ion type series and $(a_i\text{-BH})^-$ ion type series) emphasized previously are compared (Scheme 2), it appears that the sodium ion is located at the central position of the DNA single strand (i.e. the fourth phosphodiester linkage position for a 7-mer). Two other daughter ions appear at *m/z* 725 and *m/z* 882, these *m/z* values corresponding to doubly charged $(w_5\text{-AH})^{2-}$ and $(w_6\text{-AH})^{2-}$ ions. Formation of such ions is not discussed because they may be produced by particular mechanisms that are in progress and should be published elsewhere [32]; however, a possible process in terms of long range fragmentation mechanisms may be proposed.

The same experimental conditions were employed on selected $dp(T)_7$ [Fig. 1(b)]. From the ESI mass spectrum, the cationized and doubly charged $*M^{2-}$ ion at *m/z* 1084 is isolated by broadband isolation to be submitted to a CID process through the resonant excitation mode. The fragment $*[M\text{-TH}]^{2-}$ and $*[M\text{-HPO}_3]^{2-}$ ions, produced in competition, are respectively displayed at *m/z* 1020 and *m/z* 981 on the CID spectrum, according to a weak abundance as shown Fig. 1(b). This behaviour is explained by considering the frequency amplitude that allows decomposition into complementary w_i^- and $(a_{n-i}\text{-TH})^-$ ions. Moreover, the cationized fragment $*w_5^-$ and $*w_4^-$ ions are displayed at *m/z* 1560 and *m/z* 1255, whereas the diagnostic w_3^-, w_2^-, and w_1^- fragment ions are respectively observed at *m/z* 929, *m/z* 625, and *m/z* 321. For the fragment $(a_i\text{-BH})^-$ ion type, $*(a_4\text{-TH})^-$ and $*(a_5\text{-TH})^-$ ions at *m/z* 1111 and *m/z* 1415 are cationized species whereas a_1^-, a_2^-, $(a_2\text{-TH})^-$, and $(a_3\text{-TH})^-$ ions (here a_1^- and a_2^- are achieved because the TH loss is highly unfavored [31]) observed at *m/z* 303, *m/z* 607, *m/z* 481, and *m/z* 785, do not contain any sodium cation. The results of these studies show that the sodium cation is located at the fourth phosphodiester group (Table 1), still at the central position of the oligonucleotide strand.

To demonstrate the role played by the 5′-phosphorylation modification of the oligonucleotide, CID

Table 1
Sodium cation location using the diagnostic w_i^{n-}, $*w_i^{n-}$, $(a_i\text{-BH})^{n-}$ and $*(a_i\text{-BH})^{n-}$ ions of $dp(A)_7$, $dp(T)_7$, d(TTGGCCAA), d(TTAGCTAA), d(ACCCAAACCAA), and d(TTGGTTTGGGT)

	$dp(A)_7$	$dp(T)_7$	d(TTGGCCAA)	d(TTAGCTAA)	d(ACCCAAACCAA)	d(TTGGTTTGGGT)
Fragment w_i^{n-} or $(w_i\text{-BH})^{n-}$ ions	w_1^- w_2^- w_3^-	w_1^- w_2^- w_3^-	w_2^- w_3^-	w_3^-	w_1^- w_2^- w_3^- w_4^-	w_2^- w_3^-
Fragment $*w_i^{n-}$ ions	$*w_4^-$ $*w_5$	$*w_4^-$ $*w_5^-$	$*w_4^-$ $*w_5^-$	$*w_4^-$ $*w_5^-$	$*w_5^-$ $*w_8^{2-}$ $*w_9^{2-}$ $*w_{10}^{3-}$	$*w_7^{2-}$ $*w_8^{2-}$
Fragment a_i^{n-} or $(a_i\text{-BH})^{n-}$ ions	$(a_2\text{-AH})^-$ $(a_3\text{-AH})^-$	$(a_1)^-$ $(a_2)^-$ $(a_2\text{-TH})^-$ $(a_3\text{-TH})^-$	$(a_3\text{-GH})^-$ $(a_4\text{-GH})^-$	$(a_3\text{-AH})^-$ $(a_4\text{-GH})^-$	$(a_2\text{-CH})^-$ $(a_3\text{-CH})^-$ $(a_4\text{-CH})^-$ $(a_5\text{-AH})^-$	$(a_3\text{-GH})^-$ $(a_4\text{-GH})^-$
Fragment $*(a_i\text{-BH})^{n-}$ ions	$*(a_4\text{-AH})^-$ $*(a_5\text{-AH})^-$ $*(a_6\text{-AH})^-$	$*(a_4\text{-TH})^-$ $*(a_5\text{-TH})^-$	$*(a_5\text{-CH})^-$ $*(a_6\text{-CH})^-$ $*(a_7\text{-AH})^-$ $*(a_7\text{-AH})^{2-}$	$*(a_5\text{-CH})^-$ $*(a_7\text{-AH})^-$ $*(a_7\text{-AH})^{2-}$	$*(a_6\text{-AH})^{2-}$ $*(a_7\text{-AH})^{2-}$ $*(a_8\text{-CH})^{2-}$ $*(a_9\text{-CH})^{2-}$ $*(a_{10}\text{-AH})^{2-}$	$*(a_8\text{-GH})^{2-}$ $*(a_9\text{-GH})^{2-}$ $*(a_{10}\text{-GH})^{2-}$
Position of the Na^+ cation	(PO_3^-) number 4	(PO_3^-) number 4	(PO_3^-) number 5	(PO_3^-) number 5	(PO_3^-) number 6 or number 7	(PO_3^-) numbers 5 or 6 or 7 or 8, not unambiguously defined

The notation w_i^{n-} corresponds to $(w_i\text{-}n\text{H})^{n-}$ and $*w_i^{n-}$ corresponds to $[w_i\text{-}(n+1)\text{H} + \text{Na}]^{n-}$.

spectra of the isobaric $*M^{2-}$ ions, *m/z* 1213, of d(TTGGCCAA) and d(TTAGCTAA) are performed.

For the d(TTGGCCAA) w_i^- ion type, $*w_5^-$ and $*w_4^-$ ions displayed at *m/z* 1572 and *m/z* 1242, in the CID spectrum [Fig. 2(a)], are cationized singly charged ions, and the free-of-cation w_2^- and w_3^- ions are observed at *m/z* 644 and *m/z* 932 (within weak abundances). Furthermore, the $(a_i\text{-BH})^-$ ion type has been interpreted to confirm the sodium cation location on the oligonucleotide sequence. The cationized fragment $*(a_7\text{-AH})^-$, $*(a_6\text{-CH})^-$, and $*(a_5\text{-CH})^-$ ions are, respectively, at *m/z* 1964, *m/z* 1674, and *m/z* 1384, whereas the sodium-free $(a_4\text{-GH})^-$ and $(a_3\text{-GH})^-$ ions are at *m/z* 1034 and *m/z* 705. It can be concluded from these results that the position of the sodium cation is at the fifth phosphodiester bond of the 8-mer, so it is on the central position for this oligonucleotide (Table 1).

A similar behavior is observed from doubly charged $*M^{2-}$ ions of d(TTAGCTAA) and d(TTGGCCAA) (Table 1), which yield the same ion series, except the fragment $*(a_6\text{-TH})^-$ ion that is not observed in the case of d(TTAGCTAA), because the loss of TH is highly disfavored, as noted previously [32,33] [Fig. 2(b)]. These fragment ions are $*w_5^-$ and $*w_4^-$, $*(a_7\text{-AH})^-$, and $*(a_5\text{-CH})^-$, respectively, at *m/z* 1587 and *m/z* 1258, *m/z* 1963 and *m/z* 1370. Noncationized fragment ions, w_3^-, $(a_4\text{-GH})^-$, and $(a_3\text{-AH})^-$, are also observed at *m/z* 947, *m/z* 1018, and *m/z* 705. Note that the cationized $*(a_7\text{-AH})^-$ ion (*m/z* 1963–1964) is accompanied by both the 8-mer and by a doubly charged $*(a_7\text{-AH})^{2-}$ (*m/z* 980–981). The origin of this phenomenon is reported elsewhere [32].

This points out that the location of the sodium cation at the central position of the strand does not appear to depend upon the presence of a 5′-dephosphorylation at the sequence end.

In the study by Clemmer et al. [14,15], cross sections were determined for cationized negative ions of oligonucleotide. From their values it is showed that the cationized ions are more compact than the free-of-cation ones. Following the charge state, the ion adopts a compact conformation for low charge states and a linear conformation for high charge states. Thus the location of the sodium cation at the center of the oligonucleotide could lead to a folded conformation

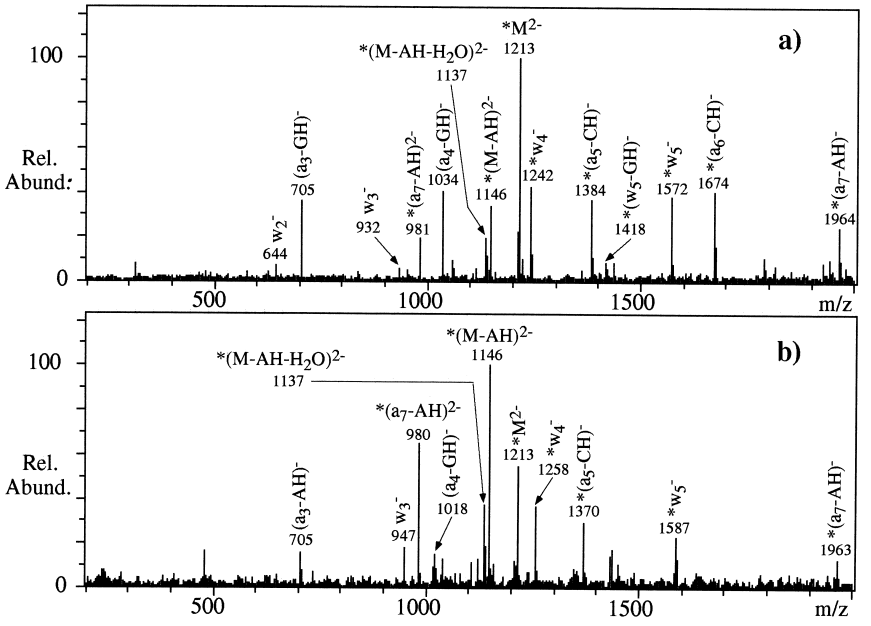


Fig. 2. CID spectra of negative ions prepared under ESI conditions, under a capillary exit voltage of −200 V, and a low *m/z* cutoff value of 300 Th, recorded after ion selection of (a) *m/z* 1213 ion ($*[M]^{2-}$) of d(TpTpGpGpCpCpApA), with a *m/z* window of 6 Th and an ion excitation with a resonant frequency amplitude of 1.0 V and a low *m/z* cutoff value of 337 Th; (b) *m/z* 1213 ion ($*[M]^{2-}$) of d(TpTpApGpCpTpApA), with a *m/z* window of 6 Th and an ion excitation with a resonant frequency amplitude of 1.0 V and a low *m/z* cutoff value of 337 Th, to be to similar q_z.

held up by hydrogen bonds, Van der Waals forces, and electrostatic interactions. For instance, the Na^+ cation at the center of the strand and the two residual charges of the phosphodiester groups at the end of the strand [32], could be in attractive interactions for maintaining a compact conformation.

It can be noticed that the size of the fragment ions is important to interpret the relative abundance of cationized or noncationized negative ions. For instance, fragmentations of the $*M^{2-}$ ion of d(TTGGCCAA) [Fig. 2(a)] lead to a series of $(a_i\text{-BH})^-$ ion types (Table 1). For each of these ions the probability of preserving or not preserving the sodium cation must be considered as a function of the number of phosphodiester linkages into the considered fragment ion. The $*M^{2-}$ parent ion contains, along the chain, seven phosphodiester linkages where seven oxygen atoms (PO^-) are partially neutralized by four protons and one sodium cation.

From this point of view, the $(a_i\text{-BH})^-$ ions are formed from the dissociation of the $*M^{2-}$ parent ion, leading to losses carrying out a number of «PO^-» and a number of counter ions (a proton or the sodium cation). The $(a_i\text{-BH})^-$ ions (i = 3–6) are formed by a cleavage involving a loss of ($8\text{-}i$) «PO^-» and ($7\text{-}i$) counter ions. This can be considered a randomization process [33], with an a priori atom scrambling number of five with a formula [Na + 4H]. If the cation attachment is not located at a defined position (i.e. at any phosphodiester linkage), the probabilities of observing (or not) the cation attached at the considered fragment ions is given by statistical considerations (Table 2).

From the CID spectrum of the $*M^{2-}$ ion of d(TTGGCCAA) [Fig. 2(a)], each $(a_i\text{-BH})^-$ and/or $*(a_i\text{-BH})^-$ ion type appears under the similar relative intensities (≈40%). From the comparison of this CID spectrum and Table 2, it appears that the location of the sodium cation in the parent ion is not statistical. Indeed, if this was the case, both the $(a_i\text{-BH})^-$ and

Table 2
Calculated probabilities to obtain, with (or without) the sodium cation, the fragment $(a_i\text{-BH})^-$ (or $*(a_i\text{-BH})^-$) ion from the parent $*M^{2-}$ ion of d(TTGGCCAA). $*M^{2-}$ corresponds to the doubly charged ion, where the four protons and the sodium cation are the counter ions. Each $(a_i\text{-BH})^-$ ion type is considered a fragment ion containing a number of retained phosphodiester linkages (PO^-), and a number of lost counter ions (n) in comparison to the parent ion

Fragment ion (i)	Number of phosphodiester linkages retained (PO^-)	Number of counter ions lost as neutral from the parent ion (n)	Probabilities to obtain the $(a_i\text{-BH})^-$ ion	Probabilities to obtain the $*(a_i\text{-BH})^-$ ion
6	5	1	0.2	0.8
5	4	2	0.4	0.6
4	3	3	0.6	0.4
3	2	4	0.8	0.2

$*(a_i\text{-BH})^-$ ions should be produced following the statistical probabilities, which is not the present case.

The last experimental series concerns the decomposition of 11-mer (d(ACCCAAACCAA) and d(TTGGTTTGGGT)), in order to test the influence of oligonucleotide length on the sodium location on triply charged $*M^{3-}$ ions. Because these oligonucleotides are characterized by a molecular weight higher than 3000 u, only the triply charged ions can be analyzed in the standard mode (50–2000 Th). These $*M^{3-}$ ions are observed on ESI mass spectra at *m/z* 1095 for d(ACCCAAACCAA) and *m/z* 1143 for d(TTGGTTTGGGT) (a shift of 7 Th for a triply charged ion containing one sodium). On the CID spectrum of the $*M^{3-}$ ions of d(ACCCAAACCAA) [Fig. 3(a)], the cationized fragment $*w_{10}^{3-}$, $*w_9^{2-}$, $*w_8^{2-}$, and $*w_5^-$ ions are displayed at *m/z* 1016, *m/z* 1380, *m/z* 1235, and *m/z* 1557, respectively. In the same way, $*(a_{10}\text{-AH})^{2-}$, $*(a_9\text{-CH})^{2-}$, $*(a_8\text{-CH})^{2-}$, $*(a_7\text{-AH})^{2-}$, and $*(a_6\text{-AH})^{2-}$ ions are observed, respectively, at *m/z* 1409, *m/z* 1264, *m/z* 1119, *m/z* 963, and *m/z* 806. In addition, the sodium-free w_4^-, w_3^-, w_2^-, and w_1^- ions are detected at *m/z* 1221, *m/z* 932, *m/z* 643, and *m/z* 330, and the fragment $[(a_5\text{-AH})^-$, $(a_4\text{-CH})^-$, $(a_3\text{-CH})^-$, and $(a_2\text{-CH})^-]$ ions are displayed at *m/z* 1278, *m/z* 989, *m/z* 699, and *m/z* 410. These results imply that the sodium cation is located at the sixth or the seventh phosphate group of the strand (according to similar probabilities), and once more at the central position of the oligonucleotide chain (Table 1). Two other ions appear at *m/z* 466 and *m/z* 767, corresponding to w_3^{2-} and w_5^{2-} ions and are not considered because their formation is not yet explained.

The same CID experiment performed on a cationized triply charged $*M^{3-}$ ion of d(TTGGTTTGGGT) [Fig. 3(b)] also suggests that the sodium cation is maintained at the middle of the DNA single strand (Table 1). However, this is not confirmed because less extensive fragmentations are observed in this case, always because the TH loss is relatively weaker than AH, CH, and GH ones. This does not allow the production of consecutive 3′ C–O bond cleavage, leading to structural ion information and particularly the cation location. This occurs even if the resonant excitation frequency amplitude is increased because this ion manipulation may lead to the loss of the fragment ions from the ion trap.

4. Conclusion

In this work, deprotonated and monocationized single-strand oligonucleotides of short length (constituted by less than 12 bases), externally prepared under nanoelectrospray conditions in the negative ion mode and injected into an ion trap mass spectrometer, have been investigated to describe and understand the fragmentations of monocationized quasimolecular anion species. Particularly, with respect toward dissociation, those induced by resonant excitation after their selection, have been examined in order to understand fragmentation according to the number of phosphate residues relative to the number of bases.

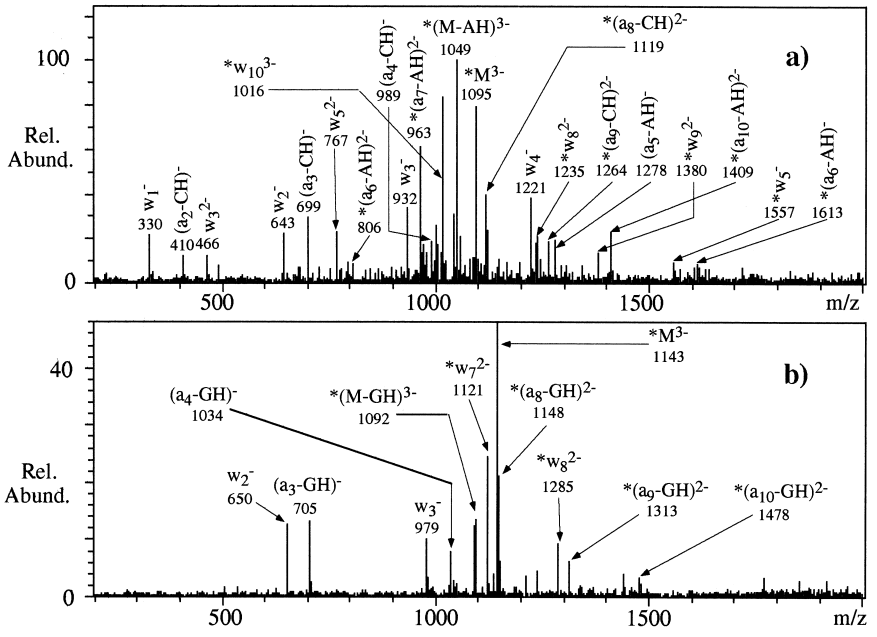


Fig. 3. CID spectra of negative ions prepared under ESI conditions, under a capillary exit voltage of −150 V, and a low *m/z* cutoff value of 200 Th, recorded after the ion selection of (a) *m/z* 1095 ion (*[M]$^{3-}$) of d(ApCpCpCpApApApCpCpApA), with a *m/z* window of 4 Th and an ion excitation with a resonant frequency amplitude of 1.1 V and a low *m/z* cutoff value of 304 Th; (b) *m/z* 1143 ion (*[M]$^{3-}$) of d(TpTpGpGpTpTpTpGpGpGpT), with a *m/z* window of 4 Th and an ion excitation with a resonant frequency amplitude of 1.1 V and a low *m/z* cutoff value of 317 Th, to be to similar q_z.

Analysis of the complementary diagnostic peaks (e.g. $[a_i\text{-BH}]^-$ as well as w_i^- fragment ions associated to their respective complementary cationized fragment ions) allows the location of the sodium cation on a particular phosphodiester position. In particular, it appears that from specific deprotonated cationized molecule dissociations of two homopolymers (e.g. $dp(T)_7$ and $dp(A)_7$), containing seven bases and phosphate groups, the alkali cation is found to be located at the fourth phosphodiester «P_4» linkage of the strand (Table 1 and Scheme 2). Similar results are provided from dissociation of two heteropolymers constituted by eight bases and seven phosphate linkages. Such a trend demonstrates that the position of the cation (i.e. at the central skeleton position) depends upon the number of phosphate units rather than the number of bases. For instance, both the «P_6» and «P_7» phosphodiester linkages are involved in the cation attachment of a single-strand oligonucleotide constituted by eleven bases and ten phosphate sites (Table 1).

Furthermore, the nature of the 5′- or 3′-residue of the studied oligonucleotides does not influence the position of the sodium cation because this position is always maintained, as shown by analysis of CID spectra of quasimolecular species. From analysis of the multiply charged fragment anions, a similar conclusion is reached concerning the position of the alkali cation. So, this means that the cation is unable to migrate from a central position to other positions closer to terminal sites. Very likely, this process has a high critical energy in spite of the use of an ion trap mass spectrometer, where the kinetic shift is relatively weak compared to those obtained by the use of a sector instrument or a tandem triple quadrupole. On the other hand, this means that the negative charge(s) of the deprotonated cationized oligonucleotides should be located at the terminal position of the strand and cannot migrate by an internal Na^+ moving [32]. However, it is not possible to rule out the eventuality in which an internal proton migration to the negative

end site takes place during the dissociation processes. It is likely that from such negative charge positions, double charge repulsion potential is restrained; this may lead by coiling to the stabilization of the conformation, which is not the case for a negative charge configuration, where the sodium salt is located at the terminal position and the negative charges are confined at an internal location.

Acknowledgements

The authors thank the Centre d'Etudes du Bouchet, University of Pierre et Marie Curie and CNRS for financial support. The technical support of BRUKER is acknowledged gratefully.

References

[1] C.J. McNeal, S.A. Narang, R.D. Macfarlane, H.M. Hsiung, R. Brousseau, Proc. Natl. Acad. Sci. USA 77 (1980) 735.
[2] C.J. McNeal, R.D. Macfarlane, J. Am. Chem. Soc. 103 (1981) 1609.
[3] L. Grotjahn, R. Franck, H. Blöcker, Int. J. Mass Spectrom. Ion Phys. 46 (1983) 439.
[4] R.L. Cerny, K.B. Tomer, M.L. Gross, L. Grotjahn, Anal. Biochem. 165 (1987) 175.
[5] D.R. Phillips, J.A. McCloskey, Int. J. Mass Spectrom. Ion Processes 128 (1993) 61.
[6] M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, Int. J. Mass Spectrom. Ion Processes 78 (1987) 53.
[7] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science 246 (1989) 64.
[8] J.P. Barry, P. Vouros, A. Van Schepdael, S.J. Law, J. Mass Spectrom. 30 (1995) 993.
[9] E. Strittmatter, P. Schnier, E. Williams, Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, FL, June 1998, p. 89.
[10] A. Liguori, A. Napoli, G. Sindona, Rapid Commun. Mass Spectrom. 8 (1994) 89.
[11] M.T. Rodgers, S. Campbell, E.M. Marzluff, J.L. Beauchamp, Int. J. Mass Spectrom. Ion Processes 148 (1995) 1.
[12] M.T. Rodgers, S. Campbell, E.M. Marzluff, J.L. Beauchamp, Int. J. Mass Spectrom. Ion Processes 137 (1994) 121.
[13] K.B. Green-Church, P.A. Limbach, Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, FL, June 1998, p. 247.
[14] D.E. Clemmer, M.F. Jarrold, J. Am. Chem. Soc. 32 (1997) 577.
[15] C.S. Hoaglund, Y. Liu, A.D. Ellington, M. Pagel, D.E. Clemmer, J. Am. Chem. Soc. 119 (1997) 9051.
[16] Y. Liu, S.J. Valentine, A.E. Counterman, C.S. Hoaglund, D.E. Clemmer, Anal. Chem. 69 (1997) 728A.
[17] J. Ni, S.C. Pomerantz, J. Rozenski, Y. Zhang, J.A. McCloskey, Anal. Chem. 68 (1996) 1989.
[18] A. De Nino, A. Liguori, L.M.T. Mario, A. Procopio, G. Sindona, J. Am. Soc. Mass Spectrom. 8 (1997) 1257.
[19] D.P. Little, R.A. Chorush, J.P. Speir, M.W. Senko, N.L. Kelleher, F.W. McLafferty, J. Am. Chem. Soc. 116 (1994) 4893.
[20] D.P. Little, D.J. Aaserud, G.A. Valaskovic, F.W. McLafferty, J. Am. Chem. Soc. 118 (1996) 9352.
[21] S.A. McLuckey, G.J. Van Berkel, G.L. Glish, Proceedings of the 39th ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, TN, May 1991, p. 981.
[22] S.A. McLuckey, G.J. Van Berkel, G.L. Glish, J. Am. Soc. Mass Spectrom. 3 (1992) 60.
[23] S.A. McLuckey, S. Habibi-Goudarzi, J. Am. Chem. Soc. 115 (1993) 12 085.
[24] S.A. McLuckey, S. Habibi-Goudarzi, J. Am. Soc. Mass Spectrom. 5 (1994) 740.
[25] N. Yates, D. Kottmeier, J. Shabanowitz, D. Hunt, Proceedings of the 42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, May 1994, p. 212.
[26] J.N. Louris, R.G. Cooks, J.E.P. Syka, P.E. Kelley, G.C. Stafford, J.F.J. Todd, Anal. Chem. 59 (1987) 1677.
[27] M.S. Wilm, M. Mann, Int. J. Mass Spectrom. Ion Processes 136 (1994) 167.
[28] R. Korner, M. Wilm, K. Morand, M. Schubert, M. Mann, J. Am. Soc. Mass Spectrom. 7 (1996) 150.
[29] I.A. Kaltashov, V.M. Doroshenko, R.J. Cotter, Proteins: Struct., Funct., Genet. 28 (1997) 53.
[30] Y. Wang, M. Schubert, J. Franzen, Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, OR, May 1996, p. 131.
[31] G. Zon, W.J. Stec, Oligonucleotides and Analogs: A Practical Approach, Oxford University Press, New York, 1991.
[32] A. Favre, F. Gonnet, J.C. Tabet (unpublished).
[33] R.G. Cooks, J.H. Beynon, R.M. Caprioli, G.R. Lester, Metastable Ions, Elsevier, New York, 1973.