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Electrospray droplet exposure to gaseous acids for reduction of metal counter-ions in nucleic acid ions

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ABSTRACT

Electrospray ionization (ESI) of oligonucleotides in either polarity mode often yields a distribution of ions at each charge state that vary in the number and identities of metal counter-ions present, thereby complicating the appearance of the mass spectrum. A variety of approaches designed to treat or modify the solution subjected to ESI have been introduced to deal with this issue. A new procedure has been developed in which chemical vapors are introduced into a region prior to the atmosphere/vacuum interface of a QqTOF instrument along with the usual counter-current nitrogen gas intended to facilitate droplet desolvation. Acidic vapors can be introduced to shift the charge state distribution of samples subjected to either positive or negative nanoelectrospray ionization as well as to reduce the number of metal counter-ions observed in DNA, siRNA and LNA ions. To remove the metal counter-ions and improve the signal levels when ionizing in the negative nanoelectrospray mode, vapors of weak acids such as acetic acid and formic acid can be mixed with the curtain gas and introduced in front of the instrument interface. For positive nanoelectrospray generated ions, the introduction of strong acids such as hydrochloric acid and trifluoroacetic acid helps to reduce the metal counter-ion incorporation and results in an increase in signal levels. The vapor addition approach is not equivalent to adding acid directly in the solution as it preserves non-covalent interactions, as seen with the preservation of the siRNA duplex during acid vapor introduction into the interface.

1. Introduction

Electrospray ionization mass spectrometry (ESI-MS) [1] has become a standard method for the mass determination of oligonucleotides [2]. Under most solution conditions, oligonucleotides are fully ionized due to the high acidities of the phosphodiester linkages. Counter-ions present in solution condense onto the oligomer in the electrospray desolvation process to neutralize some of the anionic sites. The presence of metal cations, such as sodium and potassium cations, in addition to ions that can transfer a proton to a phosphodiester linkage, such as ammonium ions, typically leads to a distribution of pseudo-molecular ions for a given charge state, varying in the amount and type of metal counter-ions that are observed, e.g., $[M-nH]^{n-}$, $M-(n+1)H+Na/K]^{n-}$, ... $[M-(n+m)H+m(Na/K)]^{n-}$ [2]. The strong electrostatic binding of the counter-ions to the oligonucleotide backbone makes them difficult to remove once the ions are in the gas-phase. The presence of mixtures of counter-ions complicates mass determination and dilutes the signal over multiple peaks. Structural characterization via tandem mass spectrometry can be compromised both due to reduced precursor ion signal strengths for a given counter-ion composition and to the fact that spectral interpretation is complicated when one or more metal ions are present in the ion. Furthermore, mixture analysis is compromised by the increased number of peaks per component.

A variety of approaches have been described to deal with the well-known counter-ion problem in the ESI-MS of oligonucleotides. For example, one method consists of adding ammonium containing salts, such as triethylammonium [3] or ammonium acetate [4], which replace metal cations in solution with the ammonium ion or protonated amine. The ammonium ion or protonated amine then dissociates into NH_3 or an amine and H^+ during the desolvation process. Other approaches include the use of ion exchange resins [5], reversed phase high-performance liquid chromatography [6], the use of sequestering agents [7,8], ethanol precipitation [3], ultrafiltration and microdialysis [9,10]. The co-addition of piperidine and imidazole results in the suppression of alkali adduct formation and causes a slight shift of the ion charge states to a higher mass-to-charge ratio [11]. These methods all require the solution to be altered and may have deleterious effects if not applied properly, resulting in the unnecessary waste of sample. Some of these techniques may also disrupt non-covalent interactions that

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may alter experimental outcomes depending on the nature of the study [12]. It is desirable, therefore, to be able to reduce or remove metal counter-ions without altering the solution subjected to ESI.

The electrospray process entails several steps involved in the transfer of ions from the solution to the gas-phase [1,13]. Isolated droplets are formed that possess a net charge and are therefore enriched at the surface in ions of the same polarity as the droplet. The droplets shrink via the evaporation of the solvent until they reach Rayleigh instability, at which point they undergo asymmetric fission. The small highly charged droplets formed in this process desolvate further and undergo subsequent fission events. This ultimately leads to the formation of very small droplets from which gas-phase ions are generated. The overall process tends to lead to a concentration of non-volatile salts such that the concentration of metal ions is increased relative to their concentration in the original solution. However, it is the concentration of the counter-ions at the surface of the fissioning droplet that is most important for the ions that ultimately appear in the mass spectrum. This is because the small droplets formed from asymmetric fission tend to sample species present at the surface of the larger droplet from which they are ejected. If the concentration of the metal counter-ions present at the surfaces of fissioning droplets can be reduced, the extent of metal ion condensation onto the oligonucleotide can be reduced. The strategies mentioned above rely either on reducing metal ion concentrations in the solution subjected to ESI and/or adding relatively high concentrations of counter-ions that can transfer a proton to a phosphodiester linkage. Herein we describe an approach that affects metal counter-ion condensation after the electrospray droplets are already formed and are evaporating in the interface of the instrument. The approach involves exposing electrospray droplets to acidic vapors prior to sampling ions into the atmosphere/vacuum interface of the mass spectrometer. Recently, an ion/ion reaction has been reported to remove Na^+ adducts from oligonucleotides after the electrospray process [14] and within the mass spectrometer. The approach described here is implemented prior to sampling ions into the mass spectrometer and places no additional reaction periods into the experiment. It should be capable of being implemented with any ESI mass spectrometer. The amount of acid vapor introduced can be easily varied and, when halted, the original oligonucleotide spectrum is once again observed.

2. Experimental

2.1. Materials

Methanol, acetic acid, formic acid, hydrochloric acid and sodium chloride were purchased from Mallinckrodt (Phillipsburg, NJ). Trifluoroacetic acid was purchased from Pierce Chemical (Rockford, IL). Initial concentrations of the acids present in the reagent test tubes are 11.7 M for hydrochloric acid, 13.0 M for trifluoroacetic acid, 23.6 M for formic acid and 17.4 M for acetic acid, unless noted otherwise. The DNA 12mers (5′OH-CTTCGCGCTGTG-3′OH) and (5′OH-CTTAGCGCTAAG-3′OH), small interfering RNA (siRNA) [sense strand = 5′OH-r(UGCGACAGGAGAUAGGCUG)d(TT)-3′OH and antisense strand = 5′OH-r(CAGCCUAUCUCCUGUCGCA)d(TT)], and locked-nucleic acid gapmer (LNA) (5′OH-GGGcttcttccttattgATGG-3′OH), were custom synthesized by Integrated DNA Technologies (Coralville, IA). (For the LNA sample—the LNA nucleotides are labeled in upper case while DNA residues are labeled in lower case.)

Oligonucleotides samples were used without further purification. Oligonucleotide solutions for both positive and negative nanoelectrospray were prepared by diluting the aqueous stock solutions to ca. 50–20 μM in 20/80 (v/v) isopropanol/water. When a greater amount of Na adduction was desired, 0.5 μM NaCl was added to the oligonucleotide solutions.

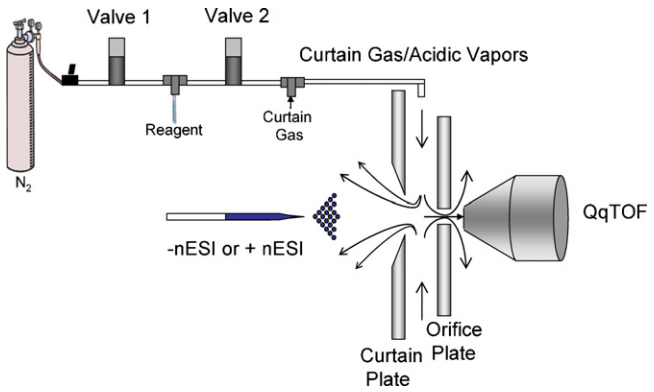

Fig. 1. Schematic of acidic vapor leak-in into a QqTOF. N_2 flow is controlled by metering valves. This flow travels across a test tube containing an acid reagent picking up acidic vapors and eventually gets mixed with N_2 curtain gas. This newly mixed flow is introduced behind the curtain plate where acidic vapors interact with nanoelectrospray generated droplets.

2.2. Apparatus and procedures

All experiments were performed using a prototype version of a QqTOF tandem mass spectrometer (Q-Star Pulsar XL, Sciex, Toronto, ON) modified to allow for ion trap CID and ion/ion reactions [15]. Ionization was accomplished via a nano-ESI emitter, forming either $[M\text{-}nH]^{n-}$ anions or $[M+nH]^{n+}$ cations of the oligonucleotides. A new apparatus was designed to allow for the introduction of acidic vapors into the interface along with the curtain gas (Fig. 1).

A Swagelok Tee connects a test tube containing approximately 20 μL of the reagent acid to a line that leads to the region between the curtain plate and orifice (nozzle) plate of the ESI interface. A flow controlled by a metering valve (Valve 1) (bellows-sealed metering valve, Swagelok, Solon, OH) of approximately 0.3 L/min of N_2 gas is directed across the test tube where head space vapors of the acid can mix with the N_2 flow. A second Swagelok metering valve (Valve 2) is located downstream from the test tube and is opened fully for most experiments, but can be used to vary the amount of N_2/acid vapors that are mixed via another Tee with the curtain gas flow (also N_2 and admitted to give a total flow of N_2/acidic vapors/curtain gas of 1.0 L/min to 1.5 L/min) before the combined flow enters the region between the curtain plate orifice and the nozzle of the ESI interface. That is, the newly mixed flow that contains acid vapors and nitrogen is introduced at slightly positive pressure between the curtain and orifice plates such that the gas blows gently out of the curtain orifice and is also drawn into the interface region (1–2 Torr) through the nozzle. This near atmospheric pressure region just outside and between the curtain and orifice plates is where the molecule/droplet interaction takes place. Interactions take place between the acid and ions in the expansion into the interface. Typically a sample is ionized via nanoelectrospray and then the metering valves are opened to introduce the acidic vapors as desired. These vapors are constantly flowing into the instrument throughout the experimental process. All acid reagents were introduced using the same flow settings during the acid comparison studies.

Gas-phase studies were performed on a modified Finnigan ion trap mass spectrometer (ITMS, Thermo Finnigan Corp., San Jose, CA) [16]. The acidic vapors were introduced after several freeze-pump-thaw cycles into the vacuum chamber through a separate inlet line using a variable leak valve (Granville Phillips, Brooks Automation Inc., Chelmsford, MA) through the same flange through which

helium bath gas is also introduced. To perform an ion/molecule reaction, nano-ESI generated anions were reacted with the leaked-in acidic vapors. The acidic vapors were introduced into the vacuum chamber to a total pressure of 2.4×10^{-4} Torr (uncorrected), as measured by an ion gauge.

Note that over time, chronic exposure to acidic vapors can lead to deleterious effects on metal components exposed to the vapors and pumping system components. We have been conducting these experiments for roughly one or two days per week over the course of roughly ten months with no noticeable effects. Evidence for TFA adduction is sometimes observed in experiments where no TFA should be present. Upon cleaning of the valves used to leak in the acid vapors, the TFA adducts are no longer observed.

3. Results and discussion

Our group has been examining various approaches to facilitate the structural characterization of 20–100mer DNA and RNA oligomers. The many phosphodiester linkages present in these molecules provide ample opportunity for metal ion adduction. Published approaches for avoiding formation of these adducts by altering solution conditions have been examined and are effective to varying degrees. However, we are also interested in the possibility of modifying the electrospray solution after the initial spray process by treating the multi-charged droplets after they are formed. Such an approach might be used alone or in conjunction with the modification of the solution subjected to ESI, as needed. In the case of the leaked vapor experiment, we noted no issues with spray formation, which can be an issue with condensed phase desalting methods. The best location to alter droplets is in the region where desolvation and Rayleigh fissioning takes place. In the present instrument, this takes place primarily in the spray plume prior to sampling into the interface region.

Acidic vapors were first leaked-in to observe the effect of the different reagents on the oligonucleotide spectra. Many acids have high vapor pressures and thus act as good reagents in our design where N_2 gas passes over the acid solution to entrain the acidic vapors prior to entering the interface. In the present arrangement, it is difficult to generate conditions where experiments with different acids yield the same gaseous acid concentrations in the interaction region. Therefore, differences in the behaviors of acids can arise from differences in concentration. However, we have noted tendencies based on acid strength, as described below. In summary, in the negative polarity, weak acids are best at removing metal counter-ions and cleaning up oligonucleotide spectra, while in the positive polarity, strong acids are best.

3.1. Negative ion polarity

A DNA 12mer, 5′-d(CTTCGCGCTGTG)-3′ was subjected to negative nanoelectrospray ionization (nESI). The observed charge state distribution (CSD) was $[M-3H]^{3-}$–$[M-5H]^{5-}$, with all of the oligonucleotide peaks containing approximately 4 Na adducts as well as other metal counter-ion adducts, thereby resulting in a poor signal-to-noise ratio (Fig. 2a and b). When acid vapors from weak acids, such as formic acid or acetic acid, were intro-

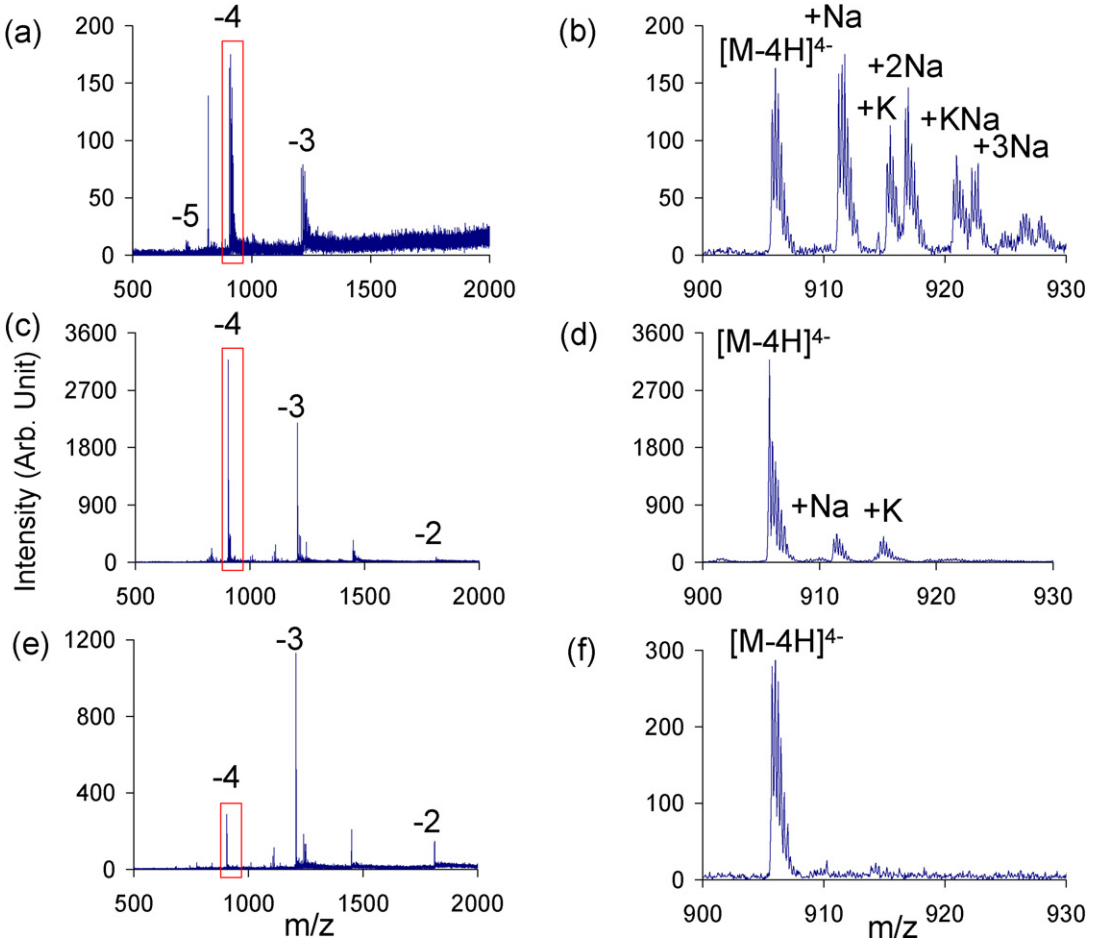

Fig. 2. Negative nESI spectrum of DNA, 5′-d(CTTCGCGCTGTG)-3′ with (a) no acid vapor, (c) acetic acid vapor, and (e) formic acid vapor. (b), (d), and (f) are zoom ins of $[M-4H]^{4-}$ adducts from (a), (c), and (e), respectively.

duced into the interface, removal of Na^+ adducts from DNA 12mer anions was observed. The dominant peak at each charge state then shifted to the unadducted $[M-nH]^{n-}$ peak. Leaking-in of acetic acid improved the signal abundance about 5× for this sample, but a slight $[M-(n+1)H+Na/K]^{n-}$ adducted peak was also observed (Fig. 2c and d). Formic acid improved the abundance by only 2× but resulted in no metal counter-ions being observed as adducts on the oligonucleotide anion peaks (Fig. 2e and f). Both acids caused a slight shift in the CSD to a lower charge state (higher m/z), so that a small $[M-2H]^{2-}$ peak was observed. Formic acid, which is a stronger acid ($pK_a = 3.75$), shifted the CSD further to lower charge states than did acetic acid ($pK_a = 4.746$) [17]. This slight shift in the CSD is similar to the one observed when these acids were added to oligonucleotides in the condensed phase [18]. In that experiment, however, no Na adduct removal was observed. Strong acids such as trifluoroacetic acid (TFA) and hydrochloric acid (HCl) were also studied as possible reagents in the negative polarity (data not shown). TFA forms adducts with oligonucleotide anions, as seen when TFA is added to oligonucleotides in the condensed phase, and was not a good reagent to reduce spectral complexity. Similarly, the introduction of HCl vapors resulted in several unidentifiable peaks and caused instability in the ion current. Favorable effects of weak acids added in the condensed phase in negative ion electrospray has been demonstrated previously, but it has not been applied in the study of oligonucleotides or in the removal of metal counter-ions [19]. The introduction of weak acid vapors proved to be best at removing Na adducts and increasing ion abundance in the negative polarity.

To explore the effectiveness of the vapor introduction procedure for a more challenging case, samples with a large excess of metal counter-ions were studied. A locked nucleic acid (LNA) gapmer (MW: 6626.7 Da), which has previously been studied in our group and desalted using traditional methods, was used [20]. (The biological relevance of this oligonucleotide has been reported by Rapozzi et al. [21].) This LNA gapmer had a charge state distribution (CSD) of $[M-6H]^{6-}$–$[M-8H]^{8-}$ with about 6 Na adducts on each charge state (Fig. 3). Ions devoid of a metal counter-ion (i.e., $[M-nH]^{n-}$ species) were largely absent in the spectrum for this sample. When acetic acid vapors were introduced, the CSD shifted two charge states to a less negative CSD $[M-4H]^{4-}$–$[M-6H]^{6-}$, as was observed with the DNA 12mer. The most dominant peak at each charge state was the unadducted $[M-nH]^{n-}$ peak. For each charge state, the percent abundance that was due to the unadducted $[M-nH]^{n-}$ peak was calculated for the sample with and without the leaking-in of acidic vapors. The leaking-in of acetic acid changed the abundance of the $[M-nH]^{n-}$ peak from 3% to 43%. The average number of Na adducts on each charge state changed from 3.6 Na to 1.2 Na.

3.2. Positive ion polarity

In the positive nESI mode, extensive Na^+ incorporation was observed for the DNA 12mer (Fig. 4). The CSD of the oligonucleotide was from $[M+3H]^{3+}$ to $[M+4H]^{4+}$. The signal-to-noise ratio was poor and adducts containing up to 3 Na ions were observed on each charge state (Fig. 4b). Often, more Na^+ adducts are observed in the positive polarity for nucleic acids because the ions observed are ultimately generated from older droplets. In positive electrospray mode, oligonucleotides are sprayed "wrong-way-round" [22,23]. Since oligonucleotides are negatively charged under most solution conditions, they tend not to compete well for surface sites at the positive droplet surface, at least for the early generation droplets. However, as the species that compete well for surface sites are concentrated in small progeny droplets and removed from larger droplets in the Rayleigh fission process, the oligonucleotides can compete better for surface sites in the later fission events of the larger droplet. The older droplets are enriched in non-volatile salts and, hence, metal ion adduction is more likely. This increased sodium incorporation has been observed when peptides and proteins were generated in the wrong-way-round condition as well [24,25]. In the positive polarity, the leaking-in of strong acids using the described procedure clearly has beneficial effects. As shown in Fig. 4c and d, the introduction of HCl increased the signal of the most abundant oligonucleotide charge state about 5-fold and improved the signal-to-noise ratio. The introduction of trifluoroacetic acid,

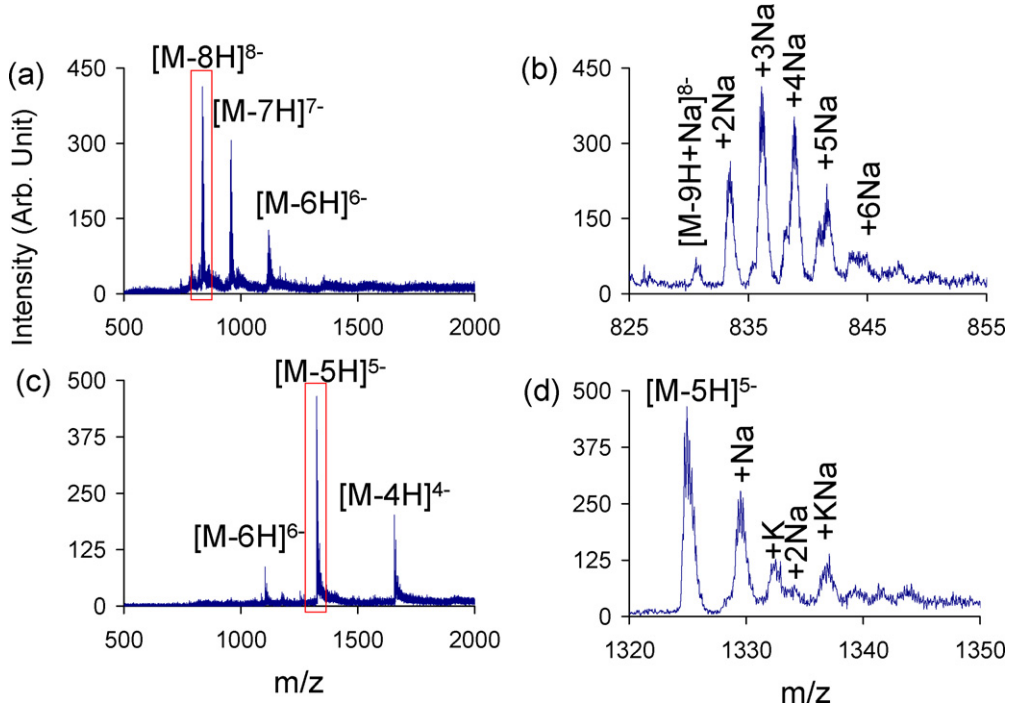

Fig. 3. Negative nESI spectrum of LNA gapmer (5′OH-GGGcttcttccttattgATGG-3′OH), with (a) no acid vapor, (c) acetic acid vapor; (b) and (d) are zoom ins of $[M-8H]^{8-}$ and $[M-5H]^{5-}$ adducts from (a) and (c), respectively. Locked bases are capitalized, while lower case letters are used for DNA bases.

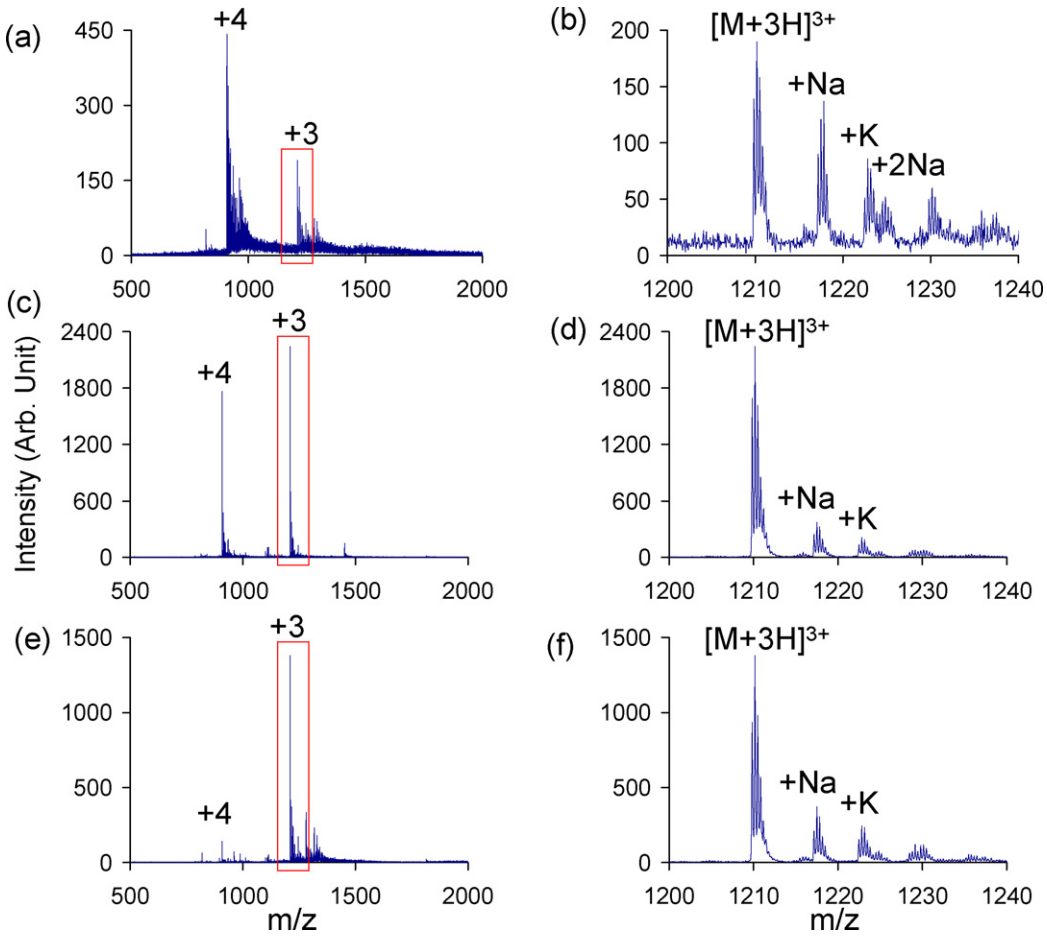

Fig. 4. Positive nESI spectrum of DNA, 5′-d(CTTCGCGCTGTG)-3′ with (a) no acid vapor, (c) HCl acid vapor, and (e) TFA acid vapor. (b), (d), and (f) are zoom ins of $[M+3H]^{3+}$ adducts from (a), (c), and (e), respectively.

another strong acid, improved the signal of the most abundant oligonucleotide charge state roughly 3-fold and also resulted in fewer metal counter-ions present on the oligonucleotide cations (Fig. 4e and f). In both cases of acid leak-in, a slight shift toward a lower CSD was observed, with the $[M+3H]^{3+}$ peak becoming the most dominant in the spectra. This shift was greater with the leak-in of TFA. Weak acids, such as acetic and formic acids, were not found to be effective at removing the metal counter-ions in the positive ion mode (data not shown), in contrast to the behavior noted in the negative mode. A slight increase in the ion abundance was observed with weak acids but this change was far less dramatic than for the strong acid case summarized in Fig. 4.

Similar to the negative polarity LNA gapmer study, a highly adducted DNA sample was studied in the positive polarity. Sodium chloride was added to a DNA 12mer (5′-d(CTTAGCGCTAAG)-3′) solution to increase the extent of Na adduction. Peaks corresponding to the +3 and +4 charge states were observed. Up to 10 Na adducts were observed on the +3 charge state (Fig. 5). Leaking-in of HCl vapors improved the abundance of the $[M+nH]^{n+}$ peak from 6% to 55% and the average number of Na adducts dropped from 4 Na adducts to 0.7 Na adducts. The leaking-in of TFA vapors increased the abundance of the $[M+nH]^{n+}$ peak to 17% and the amount of Na adducts dropped to an average of 2.5 Na adducts.

3.3. Duplexes

It is of interest to determine how exposing electrospray droplets to acidic vapors can affect the observation of ions of specific non-covalent complexes. Some of the aforementioned desalting methods have proved to be incompatible with the generation of such complexes. To address this question, a duplex formed in the positive ion mode, which is the most challenging scenario with respect to metal counter-ion condensation, was examined. A sense strand and an antisense strand of a small interfering RNA (siRNA) were annealed and ionized in the positive polarity. This siRNA sample has been previously studied in our group [26]. To get a useable siRNA anion signal with minimal counter-ion contamination, rigorous desalting procedures were required. Treatment of the sample with a cation-exchange resin was followed by the addition of piperidine/imidazole solution to the sample. Using the current procedure, no prior desalting was performed and the siRNA duplex was nanoelectrosprayed in the positive mode. As seen in Fig. 6, a relatively noisy spectrum with poor peak resolution was observed, in part due to the presence of mixtures of metal counter-ions. The signal-to-noise ratio was very poor, but peaks for the sense strand, antisense strand, and the duplex were observed. The leaking-in of HCl and TFA both helped to resolve the peaks due to the reduction of contributions of metal counter-ions. Peaks for the sense and antisense strands as well as for the duplex were now clearly observed. TFA caused a slight shift in the CSD to lower charge states, as has been observed with other positively sprayed samples. Thus, this leak-in process can lead to a removal of counter-ions and to an improvement in the signal-to-noise ratio of oligonucleotides without destroying duplexes.

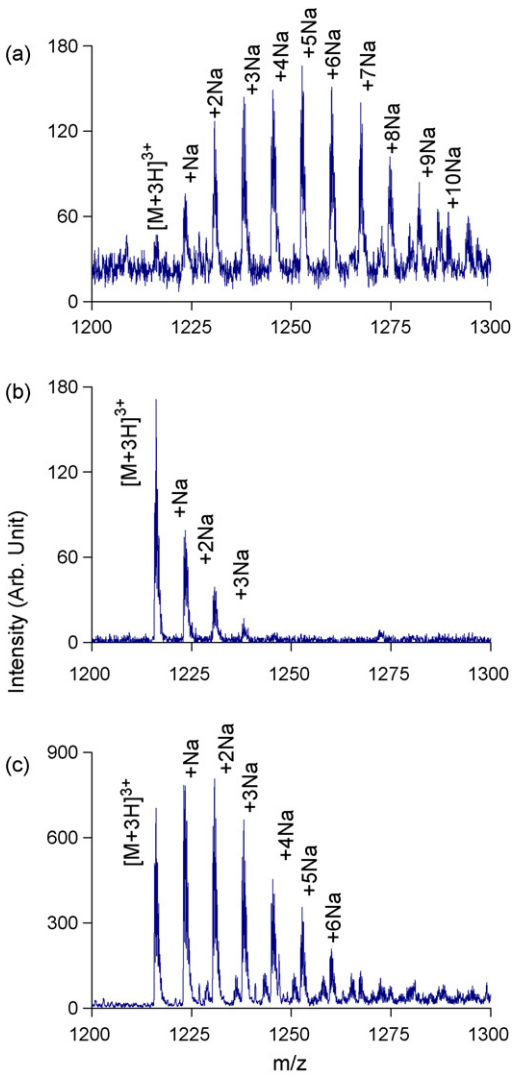

Fig. 5. Positive nESI spectrum of a NaCl adducted DNA, 5′-d(CTTAGCGCTAAG)-3′ with (a) no acid vapor, (b) HCl acid vapor, and (c) TFA acid vapor.

3.4. Mechanistic studies

ESI is a complex process with many variables that can play roles in the ultimate appearance of the mass spectrum. The detailed mechanisms, by which the exposure of ESI droplets to strong or weak acids in positive or negative ion mode affect ESI mass spectra, merit much more extensive study than has been possible since our initial observations. However, we have performed a number of experiments that shed light on the process. These studies are summarized below.

An important question is the equivalence or lack thereof of leaking-in of the acid vapor to using an ESI solution at reduced pH. In other words, how does leaking the acid into the curtain gas differ from simply adjusting the pH of the solution? Mass spectra were collected at various pH levels adjusted by the addition of HCl to solution and compared with data from the leak-in approach. In the positive mode, a reduction of counter-ions is indeed observed at reduced pH, as illustrated in Fig. 7. However, although some Na adducts are removed, the overall signal abundance decreases when acids are added in solution, which is not the case with the leak-in experiments. With the introduction of HCl vapors into the interface, the $[M+nH]^{n+}$ peak became the most intense peak (Fig. 7b). In comparison, reducing the solution pH to as low as 2 by the addition of HCl was less effective in minimizing Na adducts than the leak-in experiment. At the lowest solution pH examined, the $[M+2H+Na]^{3+}$ species gave rise to the most dominant peak (Fig. 7d). For the negative polarity, the addition of weak acids did not reduce Na adducts (data not shown). Previously, both weak and strong acids have been added to the solution phase of oligonucleotides which were then ionized in the negative polarity [18]. In that study, which focused on shifting the charge state distribution of oligonucleotides, it was also found that weak acids worked best because the stronger acids disrupted the negative ion current. Using the current method, the addition of acid occurs after the charged droplets leave the nanospray tip during the nESI process and thus the ion current is less affected.

In order to determine if the observed effects of the leak-in experiment are associated with droplets or with interactions between desolvated ions and gaseous acids, acetic acid was introduced into the vacuum chamber of a 3D ion trap mass spectrometer through a flange at the back of the instrument rather than near the interface region as in the QqTOF. Using the same LNA gapmer sample as was used on the QqTOF, no reduction in the abundance of Na adducted peaks was observed over storage times of hundreds of milliseconds when varying amounts of acetic acid were leaked into the ion trap. However, proton transfer to lower charge states (higher m/z) was observed (data not shown). This experiment suggests that this Na adduct removal is not a gas-phase phenomenon. Charge state reduction, however, can take place via ion/molecule chemistry.

The experiments just described lead us to hypothesize that the reduction of metal ion adduction in the leak-in experiments is neither a gas-phase process nor a bulk solution effect. Rather, we propose that it is the effect of the gaseous acid on the surfaces of the electrospray droplets that largely gives rise to the observed behavior. Clearly, the exposure to gaseous acids does not change the net concentration of non-volatile salts in the isolated droplets. However, the acids can affect the local environment at the droplet surface, which is a particularly important region in determining the appearance of an electrospray mass spectrum. Acid strength is an important factor and appears to have different effects depending upon droplet polarity. For negatively charged droplets, the oligonucleotides contribute negative charge to the droplet surface. The addition of a strong acid adds additional anions to the surface that can have a deleterious effect on oligonucleotide signals. Weak acids, on the other hand, do not add significant numbers of anions to the droplet surface. The intact acid can, however, engage in strong interactions with the phosphodiester linkage by engaging in proton-bound dimer formation. This interaction with the deprotonated phosphodiester linkage can impact the extent of interaction and condensation of metal ions. In the positive ion mode, strong acids can significantly alter the pH at the surface. The addition of large numbers of protons to the surface of the droplet can displace metal ions that ordinarily might occupy surface charge sites and, as a result, be available for condensation onto the oligonucleotide.

If the acid concentration at the droplet surface is a key parameter in minimizing the extent of sodium incorporation into oligonucleotide ions, the number density of the acid in the vapor phase should be an important experimental parameter. The concentration of acidic vapor present can be readily varied by changing the concentration of the acid present in the reagent test tube. The effect of varying the acid vapor concentration in the interaction region is illustrated in the positive ion mode for a DNA 12mer in Fig. 8. Results are presented for test tube HCl concentrations of 2.9 M (Fig. 8a), 5.8 M (Fig. 8c), and 8.7 M (Fig. 8e). The percent abundance of the $[M+nH]^{n+}$ peak changed from 29% to 71% as the HCl concentration in the test tube increased from 2.9 M to 8.7 M. This result suggests that the effect reported here has not reached a point of saturation. It also highlights the caution that should be taken in comparing the effects of the identities of strong acids with one another or weak

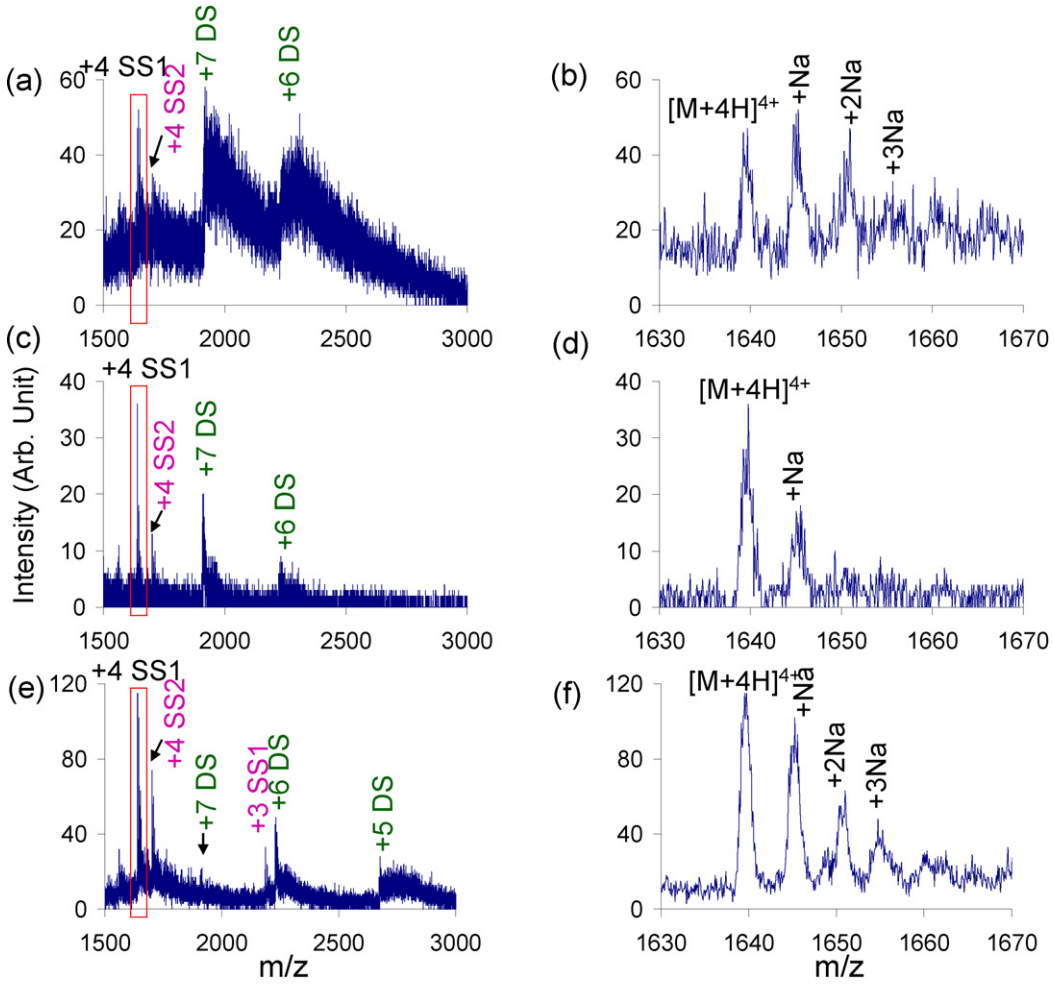

Fig. 6. Positive nESI spectrum of sense strand (SS1) 5′-r(UGCGACAGGAGAUAGGCUG)d(TT)-3′ and antisense strand (SS2) 5′-r(CAGCCUAUCUCCUGUCGCA)d(TT) annealed to make a duplex (DS) with (a) no acid vapor, (c) HCl acid vapor, and (e) TFA acid vapor. (b), (d), and (f) are zoom ins of $[M+4H]^{4+}$ adducts of SS1 from (a), (c), and (e), respectively.

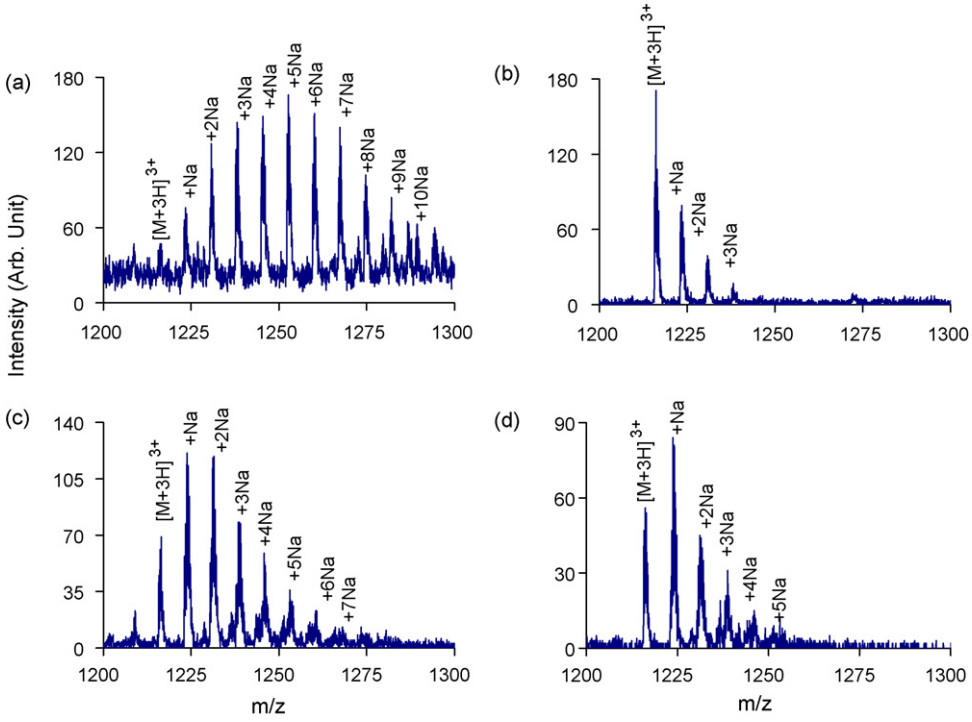

Fig. 7. Positive nESI spectrum of a NaCl adducted DNA, 5′-d(CTTAGCGCTAAG)-3′ with (a) no acid vapor, (b) HCl acid vapor, (c) HCl added to solution pH = 3 and (d) HCl added to solution pH = 2.

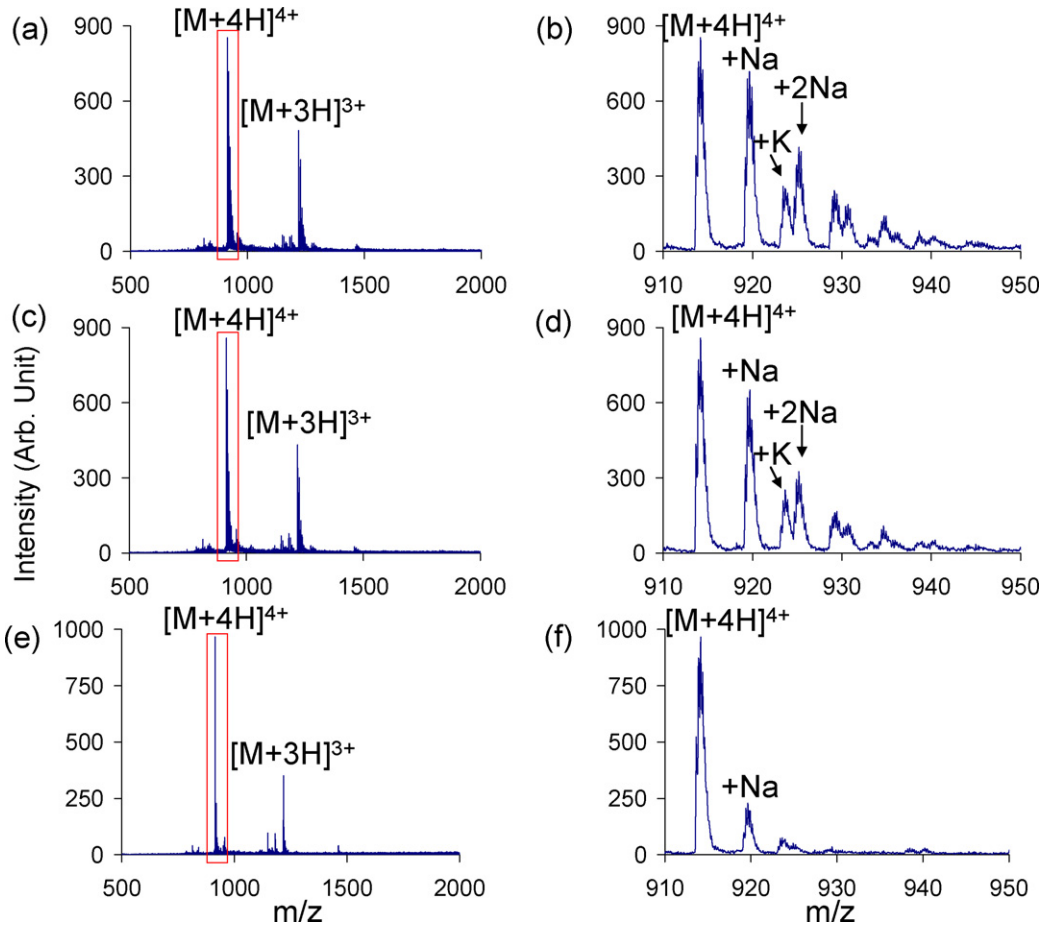

Fig. 8. Positive nESI spectrum of DNA, 5′-d(CTTCGCGCTGTG)-3 with HCl vapor leak from (a) 2.9 M HCl solution, (c) 5.8 M HCl solution, and (e) 8.7 M HCl solution. (b), (d), and (f) are zoom ins of the $[M+4H]^{4+}$ of (a), (c), and (e), respectively.

acids with one another without precise means for controlling for acid concentration.

4. Conclusions

The exposure of electrospray droplets to acid vapors can significantly affect the extent of metal counter-ion incorporation in oligonucleotide ions observed in ESI mass spectra, in both positive and negative ion polarity. This observation enables the development of a fast and facile method for affecting counter-ion incorporation that is not based on manipulation of solution conditions. In this new method, acidic vapors are introduced with the counter-current drying gas in an ESI interface, which is where the interaction occurs with the charged droplet, and leads to a reduction of metal counter-ions from the droplet surface. Oligonucleotides ionized in both the negative and positive ion polarity were studied. It was found that in the negative polarity, the introduction of weak organic acids, such as formic acid and acetic acid, increased signal abundance and reduced the presence of metal counter-ions. In the positive ion mode, the introduction of strong acids, such as hydrochloric acid and trifluoroacetic acid, worked best to clean up the oligonucleotide spectra. This phenomenon seems to be applicable with a wide range of oligonucleotides, from small 12 base long DNA molecules to larger LNA molecules and siRNA duplexes. The performance with the latter species suggests that charged droplet exposure to acidic vapors does not disrupt non-covalent complexes. The approach adds no additional time constraints and can be used by itself or in conjunction with other desalting procedures.

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