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Ammonium ion binding to DNA G-quadruplexes: do electrospray mass spectra faithfully reflect the solution-phase species?

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Abstract

G-quadruplex nucleic acids can bind ammonium ions in solution and these complexes can be detected by electrospray mass spectrometry (ESI-MS). However, because ammonium ions are volatile, the extent to which ESI-MS quantitatively could provide an accurate reflection of such solution-phase equilibria is unclear. Here we studiedfive G-quadruplexes having known solution-phase structure and ammonium ion binding constants: the bimolecular G-quadruplexes $(dG_4T_4G_4)_2$, $(dG_4T_3G_4)_2$ and$(dG_3T_4G_4)_2$, and the intramolecular G-quadruplexes $dG_4(T_4G_4)_3$ and $dG_2T_2G_2TGTG_2T_2G_2$ (thrombin binding aptamer). We found that not all mass spectrometers are equally suited to reflect the solution phase species. Ion activation can occur in the electrospray source, or in a high-pressure travelling wave ion mobility cell.When the softest instrumental conditions are used, ammonium ions bound between G-quartets, but alsoadditional ammonium ions bound at specific sites outside the external G-quartets can be observed. However, even specifically bound ammonium ions are in some instances too labile to be fully retained in the gas phase structures, and although the ammonium ion distribution observed by ESI-MS shows biases at specific stoichiometries, the relative abundances in solution are not always faithfully reflected. Ion mobility spectrometry results show that all inter-quartet ammonium ions are necessary to preserve the G-quadruplex fold in the gas phase. Ion mobility experiments therefore help assigning the number of inner ammonium ions in the solution phase structure.

Introduction

Nucleic acid sequences containing series of adjacent guanines can form particular nucleic acid structures called G-quadruplexes[1-3], in which four guanines can form G-quartets. Sufficiently high ionic strength is required to favor the self-assembly of negatively charged strands. Moreover, the very nature of the cation also influences the G-quadruplex structure and stability[4,5], because specific cation coordination occurs between the stacked G-quartets. G-quadruplex structures can differ by their strand orientation (parallel/antiparallel), by the syn/anti conformation of each base relative to the deoxyribose, and by the placement of their loops. These three structural characteristics are inter-dependent [6].

Like many other cation-bound supramolecular assemblies[7], G-quadruplexes can be studied by mass spectrometry (for a recent review, see [8]), with the restriction thatsolutions in which a physiological ionic strength (150 mM monovalent cations) would be ensured solely by Na^+ or K^+ cannot be used. Ammonium cations can be used instead[9], as NH_4^+ ions also coordinate between G-quartets and thereby stabilize G-quadruplexes in solution [10-12]. When sufficient internal energy is provided to the biomolecule, neutral ammonia is lost following proton transfer to the biomolecule. Mass spectrometry analysis of G-quadruplexes from ammonium solutions therefore entails the following tradeoff.On the one hand, it is desirable to preserve these internal ammonium cations in the gas phase, because (1) detecting them is a strong indication that G-quadruplex structures existed in solution[9], and (2) preserving them is thought to be essential to preserve the structure in the gas phase [13-15]. On the other hand, it is desirable to get rid of ammonium ions bound to less specific sites like the phosphate groups, in order to obtain cleaner mass spectraand higher sensitivity.

In parallel G-quadruplex structures, the internal ammonium ions are much less labile in the gas phase than the other ammonium ions, so mass spectral peaks with the specific number of

internal ammonium ions can be detected over a wide range of instrumental tuning settings[9,16-18]. In antiparallel structures, internal ammonium ions are usually more labile than in parallel structures [9,18], but the relative lability of inter-quartet vs. externally bound ammonium ions has not been investigated systematically. One previous MS/MS study focused on base loss and strand separation [19], which are dissociation steps subsequent to ammonium loss.

Our objective is to address the following questions in the case of antiparallel G-quadruplexes produced from ammonium acetate solutions.To what extent do the mass spectra reflect the binding constants of ammonium ions in solution? What is the optimum instrumental tuning to determine the number of specific binding sites using the ammonium ion distribution detected in the mass spectra? Would similar conclusions be reached by different users on different instruments?What is the influence of ammonium ion loss on the gas-phase structure?

To answer these questions, we studied five DNA G-quadruplexes which structures and ammonium ion binding sites have been determined by NMR in ammonium solution (Figure 1).For bimolecular G-quadruplexes $(dG_4T_4G_4)_2$(Figure 1A) and $(dG_4T_3G_4)_2$ (Figure 1B), the ammonium ion association constants are too high to be determined by NMR [20,21], so it can be considered that 100% of the G-quadruplexes contain three ammonium ions in solution. The structuraldifference lies in the placement of the loops: the T_4 loops in $(dG_4T_4G_4)_2$ are diagonal (bridging opposite corners of a G-quartet)[20], whereas the T_3 loops in $(dG_4T_3G_4)_2$ are lateral (bridging adjacent corners of a G-quartet)[21]. The G-quadruplex$(dG_3T_4G_4)_2$ (Fig. 1C) contains three G-quartets and two inner ammonium ion binding sites[22-25].Its two strands are not equivalent:one strand has a long GT_4 diagonal loop, and the other strand has one T_4 lateral loop and one propeller loop with no base.The intramolecular quadruplex $dG_4(T_4G_4)_3$ (Fig. 1D) has one diagonal T_4 loop, two lateral T_4 loops, four G-quartets and three inner ammonium ions[25,26]. Finally, the sequence $dG_2T_2G_2TGTG_2T_2G_2$ (Fig. 1E), also called the

thrombin binding aptamer (or "TBA") forms an intramolecular G-quadruplex with three lateral loops, two G-quartets and one inner ammonium ion binding site[27]. TBA binding to cations such as Na^+, K^+, Sr^{2+} has been studied by ESI-MS previously [28-30], but the detection of ammonium ion binding to TBA has not yet been reported.For the latter three sequences, the ammonium ion binding constants have been determined previously by NMR [25,27], so the theoretical distribution of ammonium ions captured between the G-quartets of the quadruplexes in solution can be calculated (Table 1).

Experimental section

The samples were prepared as described in the Supporting Information S1. Four different mass spectrometers were used, in the negative ion mode: a Solarix 9.4T ESI-Q-FTICRMS (Bruker Daltonics, Bremen, Germany), a Q-TOF Ultima Global ESI-Q-TOFMS, a Synapt G1 and a Synapt G2 HDMS ESI-Q-IM-TOFMS (the latter three from Waters, Manchester, UK). The effects of instrumental tuning on the ammonium ion preservation will be illustrated with the bimolecular quadruplex $[(dG_4T_4G_4)_2]^{5-}$ (Figure 2 and Supporting Information S2), which is particularly sensitive to instrumental tuning [9]. The softest possible conditions, taken as those giving the highest ratio between the 3-NH_4 peak and the 2-NH_4 peak, were sought on each mass spectrometer. The softest possible experimental conditions on each mass spectrometer are given in Supporting Information S2, with extended discussion of critical parameters.

Ion mobility calibration was carried out using oligonucleotides of known collision cross section (CCS), as described previously (supporting information of[31]).The prediction error (the CCS interval in which one has 95% chances of finding the true value usingthe calibration curve) is 1.6% and the confidence error (the CCS interval in which repeated measurements of

molecules belonging to the dataset will be found) is 0.3%. The prediction error must be considered when comparing our experiments with experiments carried out on a different instrument or with calculations, and the confidence error must be considered when comparing different systems measured on the same instrument. The CCS distributions were fitted using PeakFit 4.11. The instrumental ion mobility mobility resolution in the concerned *m/z* range is 22.5, determined as $\Omega/\Delta\Omega_{FWHM}$of the G-quadruplex $[(dTG_4T)_4\bullet(NH_4)_3]^{5-}$. This parallel G-quadruplex was chosen because it is very rigid[13,32,33],and its CCS peak displays a resolution of 75 on the Synapt G2 HDMS.

Results and discussion

Electrospray mass spectrometers are not equally suited to preserve specific inner ammonium ions

The Solarix ESI-FTICRMSand the Synapt G1 provided the softest experimental conditions (see Figure 2A for a typical spectrum).The two other Q-TOF type instruments were found harsher than the Synapt G1, but for different reasons.With the particular source of the Q-TOF Ultima Global mass spectrometer (Figure 2B), the 3-NH_4/2-NH_4 ratio is similar to that obtained on the ESI-FTICRMS (Fig. 2A), but simultaneously, a peak with zero ammonium ion adducts is also observed. Therefore, a major fraction of the ions is produced in soft conditions, while a small fraction of the ions acquires a significantly higher internal energy, and the whole ESI-MS ammonium ion binding distribution does not reflect the solution phase distribution.On the Synapt G2 HDMS, ammonium loss was more abundant in the ion mobility mode than on all other mass spectrometers.

Collision-induced dissociation occurs inside the travelling wave ion mobility cell

In addition to ion heating before the IMS cell[34,35], ion heating could also occur inside travelling wave IMS cells, as reported for small singly charged ions[35,36]. However, as the extent of ion heating is supposed to decrease when the size of the system increases, increase when the charge increases, and decrease when the cell pressure increases, whether detectable ion heating inside the IMS cell would be observed for larger, multiply charged ions on a Synapt G2 mass spectrometer remained unknown. For $[(dG_4T_4G_4)_2\bullet(NH_4)_3]^{5-}$, lowering the IMS wave height from 40 V to 25 V (Figures2Cand 2D, respectively), or increasing the IMS wave velocity from 1000 m/s to 1600 m/s (see Supporting Information S2) leads to better preservation of the three ammonium ions, confirming that ion heating is partly occurring inside the IMS cell.

In some G-quadruplexes, inner ammonium ions are too labile to be fully retained in the gas phase structures

For $(dG_4T_4G_4)_2$ (Fig. 2A) the bias of the ammonium ion distribution towards the 3-NH_4 species as the conditions soften is clear, but the 2-NH_4 peak remains at ~20%, for both charge states. The ammonium ammonium ion distributionsfor the other G-quadruplexes, recorded on the Solarix ESI-FTICRMS instrument in the softest possible conditions, are shown in Figure 3 (Supporting Information section S3 shows the full scan mass spectra, and zooms on the ammonium ion distribution of the two lowest charge states). For $(dG_3T_4G_4)_2$ (Fig. 3B) and $dG_4(T_4G_4)_3$ (Fig. 3C), the ammonium ion distribution mirrors the one expected from the solution phase NMR data (Table 1). The two othersequences, however, failed to give spectra close to the expected ammonium ion distribution. The mass spectra of the sequence $(dG_4T_3G_4)_2$ (Fig. 3A) show the 2-NH_4 species as the dominant peak in all conditions, for both

charge states, despite the fact that this G-quadruplex contains exclusively three ammonium ions in solution. Ammonium ion loss at some late stage of electrospray (when solvent is still present) can be ruled out, because based both on the melting temperatures and on the ammonium ion exchange rates, the solution-phase stability of bimolecular G-quadruplexes in solution ranks in the following way: $(dG_4T_3G_4)_2$>$(dG_4T_4G_4)_2$>$(dG_3T_4G_4)_2$. In the ESI-MS spectra, ammonium ion preservation ranks in the reverse order.As will be demonstrated below in the ion mobility section, ammonium ion preservation depends mainly on ammonium ion kinetic stability in the gas phase, not in solution.

ESI-MS spectra suggest additional specific ammonium ion binding sites

In some spectra, additional ammonium ions compared to the number trapped between the G-quartets can also be detected. The question is whether these extra ammonium ions are located at specific binding sites or rather bound to the phosphodiester backbones of the strands. Specific binding sites would have higher binding strength in solution than ammonium counter-ion binding to the phosphate groups. If this higher binding strength in solution translates into a higher kinetic stability in the gas phase, the mass spectra could show a bias in the ammonium ion distribution towards a particular stoichiometry higher than the number of cations trapped between G-quartets. Note that ammonium ions in these external binding sites are invisible to NMR, either because of the short residence times, or because their partial hydration is making them equivalent to bulk ammonium ions.

Thrombin binding aptamer (TBA)

The TBA intramolecular G-quadruplex (Fig. 3D)differs from the quadruplexes reported above. The peak corresponding to two ammonium ions bound is the base peak for charge state 3-. The existence of a second cation binding site for the TBA in solution is

currentlycontroversial[30,37-39]. Our mass spectrometry results clearly show the preservation of an additional specific ammonium cation by the TBA in addition to the one likely bound between the G-quartets,suggestingthat a fraction of the population in solution possessed this additional cation. Interestingly, molecular dynamics simulation of cation (Na^+ and K^+) movement in the TBA in solution showed that cations can be transiently captured between the loops before replacing the central cation [40,41]. Similar results were found in preliminary simulations made on TBA in the presence of ammonium cations in solution [A.V. Golovin, personal communication]. However, the change in ammonium ion distribution according to the charge state prevents the quantification of this species by MS. The 2:1 complex is very labile in the gas phase (see below), even for the lowest charge state, so it is also possible that the 2:1 complex is partially dissociated in the gas phase even in the softest possible conditions.

Sequences containing diagonal loops

In $dG_4(T_4G_4)_3$ (Fig. 3C), one additional ammonium ion is detected for charge state 6-, but the lack of bias towards a specific stoichiometry prevents attributing it unambiguously to a specific binding site. In contrast,in $(dG_3T_4G_4)_2$ (Fig. 3B), the peak with a total of 3 ammonium ions preserved is clearly more abundant than the peak with 4 ammonium ions. Note that for sensitivity reasons the spectrum in Fig. 3Bwas recorded with a drying gas temperature of 150°C instead of 20°C. The biased distribution towards the 3-NH_4 peak is even more clearly observed on the Q-TOF and on the Synapt G1 instruments (Supporting Information S4).The mass spectra therefore hint at the presence of one additional specific binding site. Finally, the G-quadruplex $[(dG_4T_4G_4)_2]^{5-}$ (Fig. 2) a peak with 5 ammonium ions is retained, hinting at the presence of two such additional specific binding sites. Interestingly, X-ray crystallography studies showed that, in addition to binding between G-quartets, K^+ cations can also bind to the loops of $(dG_4T_4G_4)_2$[42]. As this structure with two equivalent diagonal loops takes up two

additional ammonium ions, whereas G-quadruplexes $(dG_3T_4G_4)_2$ and $dG_4(T_4G_4)_3$ have only one diagonal loop each, we propose that outer G-quartets are additional ammonium ion binding sites and that the bases of the diagonal loops participate in the cation binding. Guanines in the loops (G-quadruplexes $(dG_3T_4G_4)_2$ and TBA) also favor additional ammonium ion binding.These results alsorecall the capture of one additional ammonium ion between the outer G-quartets and heterocyclic ligands like telomestatin [43,44]. ESI-MS is the only experimental method capable of probing these outer-quartet specific binding sites directly, and will be very useful to reveal the existence of such binding sites in new systems. However, the results will require further validation by molecular dynamics simulations or by ion titration experiments.

All inner ammonium ions are necessary to preserve the G-quadruplex fold in the gas phase

The previous ion mobility spectrometry studies already showed that the preservation of inner ammonium ions was associated with a better preservation of the solution-phase structure in the gas-phase [14]. However, the quadrupole resolution in these past studies prevented probing the effects of internal energy increase and ammonium loss individually. Here, we systematically investigated the influence of the bias voltage (injection voltage in the ion mobility cell), of the number of preserved ammonium ions, and of the charge state on the CCS. A typical driftscope spectrum is shown in Supporting Information S5. After CCS calibration, the resultsare 4D-data (mass-intensity-CCS-bias), presented for clarity in two 2D-plots. Figure 4 shows representative data, and Supporting Information S6gathers the full dataset.The lower plot shows the evolution of the CCS with the bias voltage for each mass (here, for each number of ammonium ions retained), and the upper plot shows the relative

abundance of each peak, indicating the relative gas-phase kinetic stability of ammonium ions in G-quadruplexes.

The collision cross sections of the species retaining the exact number of specific ions known to bind between G-quartets change little with the bias voltage or with the charge state. The centroid CCS of the species retaining all specific ammoniums are listed in Table 2, along with the CCS resolution calculated from the full width at half-maximum of the Gaussian peak fittings. The CCS peak widthsare wider than the instrumental resolution, and vary with the nature of the oligonucleotide, the charge state, and the voltage, suggesting that the CCS peaks correspond to a conformational ensemble rather than to a single gas-phase structure. The data in Table 2 constitute a solid basis for future work comparing experimental and calculated CCS.The Table reveals that, based on the collision cross section alone, it is problematic to assign the fold, even if it is preserved from the solution. For example, $(dG_4T_3G_4)_2$ and $(dG_3T_4G_4)_2$ have the same number of bases, completely different folds (Figs. 1B and 1C), and yet the same CCS at low charge state when retaining their specific number of ammonium ions.

For each bimolecular G-quadruplex, the loss of a single inner ammonium ion significantly changes the collision cross section. In general, in the lowest charge state the ammonium ion loss induces a slight crunch of the structure, whereas in the highest charge states it induces a dramatic expansion of the structure. For the most abundant charge state (5- for all dimers presented here), the direction of the change varies with the sequence. Moreover, the absence of change in CCS does not necessarily mean the absence of change in gas-phase structure. For example, for $[(dG_4T_4G_4)_2]^{5-}$ (Figure 4A), the first ammonium ion loss induces an expansion of the structure, then the two subsequent ammonium ion losses induce consecutive crunches of the structure. Incidentally, the 1-NH_4 CCS is equal to the 3-NH_4 CCS. Therefore, to ensure preserving the G-quadruplex structure from the solution to the gas phase, the species with all

specific inner ammonium ions and at relatively low charge state must be selected (even when this species is particularly labile, such as the 3-NH_4 species for $(dG_4T_3G_4)_2$, see Fig. 4B.

In the case of intramolecular G-quadruplexes (Supporting Information Figure S6-2), for the two lowest charge states (including the most abundant charge state), the collision cross section does virtually not change upon ammonium ion loss. Ammonium loss involves proton transfer of the ammonium ions to the DNA, and hence the repartition of the charges over the DNA structure at total constant charge changes upon ammonia loss. This apparently is not always accompanied by a change of collision cross section for the lowest charge states, presumably because other salt bridge interactions in the denatured state overcome the Coulombic repulsion and the denatured state remains compact.

Ion mobility spectrometry helps to assign the number of inner ammonium ions

On the contrary to inner ammoniums, additional ammonium ions are not essential to preserve the gas-phase structure (compare the CCS of the 3-NH_4 and 2-NH_4 species in $(dG_4T_3G_4)_2$,Figure 4B, where the three inner ammonium ions are essential,with $(dG_4T_3G_4)_2$,Figure 4C, where only two inner ammonium ions are essential).Additional (i.e., outer-G-quartet) ammonium ions cause little changes of CCS (see for example the 3-NH_4 species for $(dG_3T_4G_4)_2$ in Fig. 4C). At the highest charge state, the mean CCS and the peak width of the intact complex increase with the bias voltage, but in lower proportion than when ammonium ion loss occurs. This suggests that the solution structure is preserved in the gas phase when all inner ammonium ions are preserved. Moreover, the most significant change in CCS is observed for the highest charge state (compare Figure 4C and 4D). IMS experiments on the high charge state may not be ideal to derive structure from the CCS, but give the best indication that a specific number of ammonium ions are involved in the formation of a

specific structure like a G-quadruplex. The number of ammonium ions needed to keep the CCS of the high charge state ions close to the CCS of the lower charge state ionsis equal to the number of inner ammonium ions in the solution structure.

Conclusions

Assigning the number of specific ammonium binding sites in G-quadruplexes using electrospray mass spectrometry is difficult. First, inner ammonium ions (i.e., those residing between G-quartets) are relatively labile in the gas phase in the case of antiparallel G-quadruplexes. Second, additional specific binding sites (i.e., outside G-quartets) also exist in solution and can be preserved by ESI-MS, butthe difference in gas-phase lability between ammonium ions bound at these additional specific sites and ammonium ions non-specifically attached to phosphate groups is less substantial. To reach reliable conclusions on ammonium ion binding in solution based on ESI-MS data, we make the following recommendations:

(1) Instrument choice is crucial. A good system to test instrumental conditions is the $(dG_4T_4G_4)_2^{5-}$ bimolecular G-quadruplex, for which soft conditions should show the 3-NH_4 peak (all inner ammonium ions preserved) as the base peak. The 5-NH_4 peak (with two additional ammonium ions presumably bound between the external G-quartets and the diagonal loops) should also be visible.

(2) The gas-phase lability should be tested on a range of relative internal energies, andonly clear biases in the ammonium ion distribution can be interpreted as an indication of specific ammonium ion binding. This helps assigning the number of binding sites in solution.With regard to determination of ammonium ion equilibrium binding constants, when some lability is evident atconditions close to the softest

achievable, the ammonium ion distribution will likely not reflect the solution-phase distribution.

(3) Ion mobility spectrometry helps assigning the number of inner ammonium ions as the minimum number of ammonium ions needed to obtain a near-constant CCS at all charge states.Indeed, all inner ammonium ions are necessaryand sufficient to preserve the structure from the solution to the gas phase, for the most abundant charge state produced from solutions at physiological ionic strength (150 mM NH_4OAc).

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Tables

Table 1. Predicted distribution of ammonium ions preserved between the G-quartets of each G-quadruplex structure in solution, based on NMR data [20,21,25,27].

G-quadruplex	**0-NH_4**	**1-NH_4**	**2-NH_4**	**3-NH_4**
$(dG_4T_4G_4)_2$	0%	0%	0%	100%
$(dG_4T_3G_4)_2$	0%	0%	0%	100%
$(dG_3T_4G_4)_2$	0%	0.5%	99.5%	-
$dG_4(T_4G_4)_3$	0.2%	3.8%	29%	67%
TBA	3%	97%	-	-

Table 2. Collision cross sections and CCS peak resolution for the intact G-quadruplexes containing all inner ammonium ions.

G-quadruplex	**3-**		**4-**		**5-**		**6-**		**7-**	
	Ω (Å²) [a]	$\Omega/\Delta\Omega_{FWHM}$	Ω (Å²) [a]	$\Omega/\Delta\Omega_{FWHM}$	Ω (Å²) [a]	$\Omega/\Delta\Omega_{FWHM}$	Ω (Å²) [a]	$\Omega/\Delta\Omega_{FWHM}$	Ω (Å²) [a]	$\Omega/\Delta\Omega_{FWHM}$
$(dG_4T_4G_4)_2\bullet(NH_4)_3$			802 ± 3	17.0	801.9 ± 0.6	18.7	803.4 ± 0.4	14.9		
$(dG_4T_3G_4)_2\bullet(NH_4)_3$			740.2 ± 1.2	15.8	746.7 ± 0.7	12.2	753.2 ± 1.3	10.5		
$(dG_3T_4G_4)_2\bullet(NH_4)_2$			740 ± 3	13.6	747.3 ± 1.7	12.0	762 ± 3	10.0		
$dG_4(T_4G_4)_3\bullet(NH_4)_3$					896.6 ± 0.6	15.1	915.4 ± 1.2	12.9	940 ± 5	10.8
TBA$\bullet NH_4$	565.9 ± 1.0	11.8	583 ± 2	9.7	624.6 ± 1.7	7.5				

[a] Average values and standard deviations obtained from three measurements on the Synapt G1 HDMS at the three lowest bias voltages.The prediction error (95% confidence) is 1.6% in all cases.

Figures

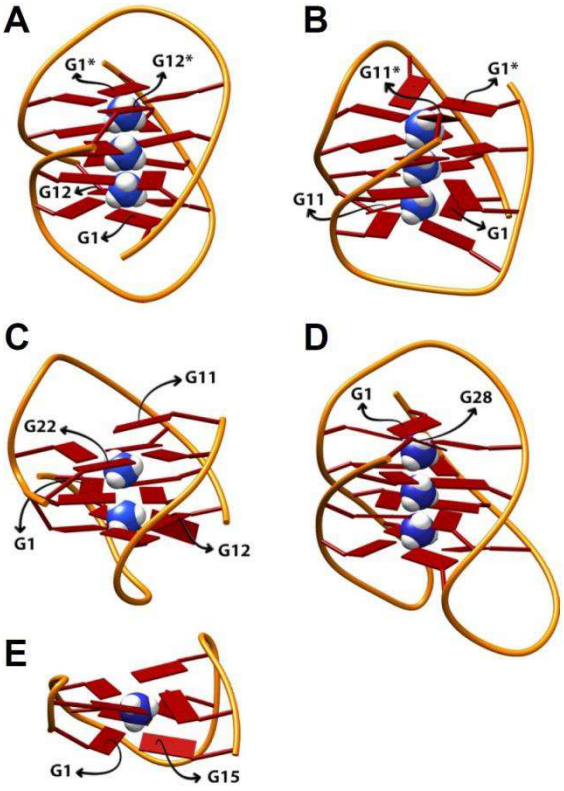


Figure 1: Structures and inner ammonium ion binding sites of the G-quadruplexes studied here: bimolecular (A) $(dG_4T_4G_4)_2$, (B) $(dG_4T_3G_4)_2$ and (C) $(dG_3T_4G_4)_2$, and intramolecular (D) $dG_4(T_4G_4)_3$ and (E) $dG_2TTG_2TGTG_2TTG_2$, also called "TBA". The 5' and 3'-ends of each strand are labeled with residue numbers. The figures were generated from PDB entries 156D (A), 2AVH (B), 1U64 (C), 201D (D) and 148D (E).

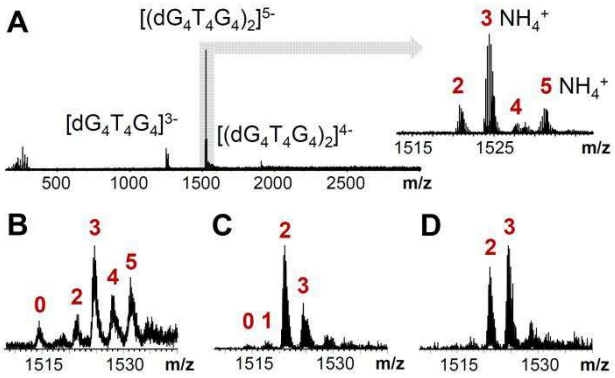


Figure 2: Influence of instrument choice and of traveling waves on ammonium ion preservation in $[(dG_4T_4G_4)_2]^{5-}$. The number of preserved ammonium ions is written in red. **(A)** Solarix mass spectrum in softest conditions. **(B)**Ammonium ion distribution obtained on the Q-TOF Ultima Global in softest conditions. **(C-D)**Ammonium ion distribution obtained on the Synapt G2 HDMS (bias of 35 V,p_{IMS} = 1.820 mbar, wave velocity = 1000 m/s), with wave height at 40 V**(C)**or 25 V **(D)**.

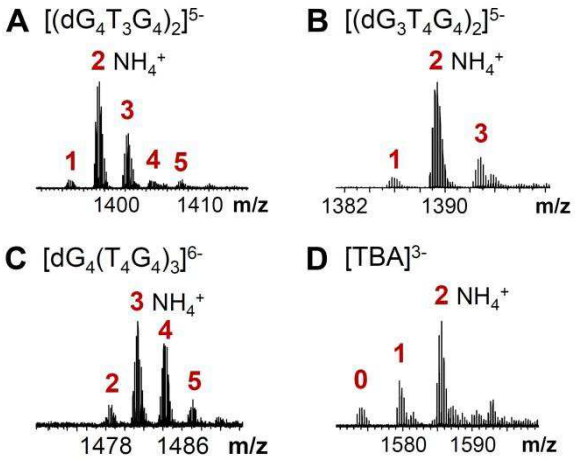


Figure 3: Ammonium ion distrubutions recordedusingsoft instrumental conditions on the Solarix ESI-FTICR instrument. Solution conditions: 100% aqueous 150 mM NH_4OAc with 10 µM DNA strand (A) $dG_4T_3G_4$, (B) $dG_3T_4G_4$ ($T_{dry\ gas}$ = 150°C), (C) $dG_4(T_4G_4)_3$ ($T_{dry\ gas}$ = 220°C), (D) $dG_2T_2G_2TGTG_2T_2G_2$ = TBA.

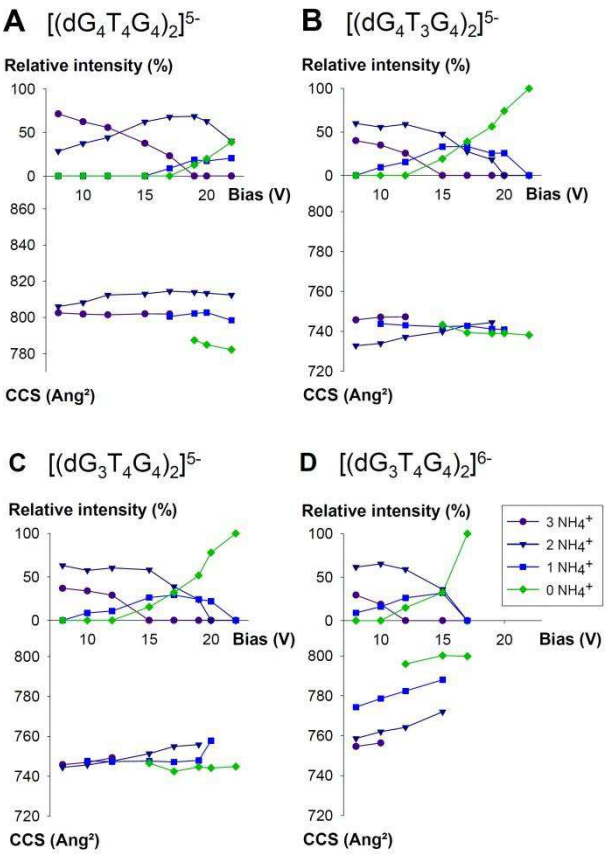


Figure 4: Influence of the bias voltage and of the number of preserved ammonium ions on the collision cross sections (CCS) of G-quadruplexes. The full dataset is in Supporting Information S6.